Vol. XIV, No. 3 August, 1927

THE ANNALS OF APPLIED BIOLOGY

EDITED FOR THE ASSOCIATION OF ECONOMIC BIOLOGISTS

BY

W. B. BRIERLEY

AND

D. WARD CUTLER

PUBLICATIONS COMMITTEE

W. L. BALLS
V. H. BLACKMAN
A. D. IMMS
R. T. LEIPER
G. A. K. MARSHALL

CAMBRIDGE UNIVERSITY PRESS
LONDON: FETTER LANE, E.C. 4
also
H. K. LEWIS & CO., LTD., 136, GOWER STREET, LONDON, W.C. 1
WHELDON & WESLEY, LTD., 2, 3, 4, ARTHUR ST., NEW OXFORD ST., W.C. 2
PARIS: LIBRAIRIE HACHETTE & CIE.
CHICAGO: THE UNIVERSITY OF CHICAGO PRESS
(AGENTS FOR THE UNITED STATES)
BOMBAY, CALCUTTA, MADRAS: MACMILLAN & CO., LTD.
TOKYO: THE MARUZEN-KABUSHIKI-KAISHA

Price Twelve Shillings net

PRINTED IN GREAT BRITAIN

The Association of Economic Biologists

President

J. C. F. FRYER, M.A.

Vice-Presidents

A. D. IMMS, M.A., D.Sc.
G. H. PETHYBRIDGE, B.Sc., M.A., Ph.D.

Hon. Treasurer

A. D. IMMS, M.A., D.Sc.,
Rothamsted Experimental Station,
Harpenden

Hon. Editors

W. B. BRIERLEY, D.Sc.
D. WARD CUTLER, M.A.,
Rothamsted Experimental Station,
Harpenden

Hon. Secretary (General and Botanical)

T. F. CHIPP, M.C., B.Sc., Ph.D.,
Royal Botanic Gardens,
Kew,
Surrey.

Hon. Secretary (Zoology)

J. WATERSTON, M.A., D.Sc.,
Nat. Hist. Museum,
South Kensington, S.W. 7

Council

Prof. B. T. P. BARKER, M.A.
Prof. V. H. BLACKMAN, Sc.D., F.R.S.
F. T. BROOKS, M.A.
N. CUNLIFFE, M.A.
G. FOX-WILSON.
T. GOODEY, D.Sc.
J. MUNRO, D.Sc.
S. G. PAINE, D.Sc.
G. H. PETHYBRIDGE, B.Sc., M.A., Ph.D.
Lt.-Col. Sir DAVID PRAIN, C.M.G., C.I.E., LL.D., F.R.S.
H. V. TAYLOR, B.Sc.
S. P. WILTSHIRE, B.Sc., B.A.

CONTENTS OF Vol. XIV, No. 3

	PAGE
1. On Forms of the Hop Resistant to Mildew (*Sphaerotheca humuli* (DC.) Burr.); VI. Temporary Loss of Immunity. By Prof. E. S. Salmon	263
2. Grafting Experiments with Varieties of Hops Resistant to the Hop Powdery Mildew, *Sphaerotheca humuli* (DC.) Burr. By Prof. E. S. Salmon and W. M. Ware. (With Plate XVIII)	276
3. Rhizoctonia "Foot-Rot" of the Tomato. By T. Small	290
4. Mangel Scab—Its Cause and Histogeny. By W. A. Millard, B.Sc., and Fred Beeley, B.Sc. (With Plates XIX–XXII and 1 Text-figure)	296
5. A Study of *Hylemyia* (*Chortophila*) *Brassicae* Bouché, the Cabbage Root Fly and its Parasites. With Notes on some other Dipterous Pests of Cruciferous Plants. By Kenneth M. Smith, D.Sc. (With Plate XXIII and 10 Text-figures)	312
6. Studies on Contact Insecticides. Part VI. The Insecticidal Action of the Fatty Acids, their Methyl Esters and Sodium and Ammonium Salts. By F. Tattersfield and C. T. Gimingham. (With 9 Diagrams)	331
7. Investigations on the Control of Wireworms. By Herbert W. Miles, M.Sc. (Bristol), N.D.A., and F. R. Petherbridge, M.A. (Cantab.). (With Plates XXIV and XXV and 2 Text-figures)	359
8. Reviews	388

ON FORMS OF THE HOP RESISTANT TO MILDEW (*SPHAEROTHECA HUMULI* (DC.) BURR.); VI. LOSS TEMPORARY OF IMMUNITY

BY PROF. E. S. SALMON.

(*Mycological Department, South-Eastern Agricultural College, Wye, Kent.*)

DURING 1926 a case was observed where three varieties of hops (*Humulus Lupulus*) which had previously shown complete resistance to the attacks of the "powdery mildew" *Sphaerotheca Humuli* lost, temporarily, this immunity. The details concerning each variety will first be given separately and then the factors possibly concerned with the loss of immunity will be considered.

VARIETY 1. THE GOLDEN HOP.

Origin. The exact origin of the Golden Hop—a horticultural variety used for decorative purposes—is unknown, but it appears to have originated on the Continent ((4), p. 293).

Evidence as to Immunity. Plants of this variety, grown in pots in an unheated greenhouse, were first tested in 1916 and were found to be completely resistant to the attacks of the mildew. Details of the experiments have already been given ((2), p. 88). Further inoculation experiments to test its immunity were carried out in 1917, and some of these, as was then stated ((2), p. 89), took place "under abnormal weather conditions, when rapid changes of temperature occurred." No infections resulted and the conclusion was reached that "the abnormal weather conditions had no effect upon the immunity" of this variety. It was again tested in 1918 and 1919; 47 plants in pots were repeatedly inoculated throughout the growing season and remained persistently immune ((4), p. 296).

Partial break-down of Immunity in 1926. In the spring of 1926, the mildew was found for the first time on the Golden Hop. The material affected consisted of (*a*) old and (*b*) young plants, and the details observed were as follows.

(*a*) By April 28th, a plant of the Golden Hop which has been grown in the greenhouse for a number of years, had produced a shoot 8·5 cm.

long, with six pairs of leaves. At this date one leaf at the 2nd node from the tip bore two minute "powdery" patches[1] of the conidial stage of *S. Humuli*, both situated on the extreme margin of the terminal lobe (one on each side) and near its base. On May 10th, by which date the shoot had not elongated, owing to the weather conditions, the two minute powdery patches were persisting but had not appreciably increased in size; a third minute, densely powdery patch had appeared on the lamina of the same leaf, at a place opposite and close to one of the sinuses at the base of the terminal lobe. Further, a very minute powdery patch was present on a leaf at the next node above.

All the above-described patches remained powdery for some weeks without noticeably increasing in size, and then slowly died away. No further infection of this plant occurred. During May and June the shoot grew to several feet in length, and other shorter shoots appeared. Although the plant was kept in the greenhouse throughout the season, exposed continually to infection by conidia blown on to it from a large number of adjacent mildew-infected hop plants, all the leaves remained healthy, just as they had invariably done in previous seasons.

Table I.

Date of exam.	Pot	Length of shoot (cm.)	No. of pairs of leaves	Condition
Apr. 28	1	15·5	6	All healthy
May 11	1	18·5	8	Two leaves, both at the 4th node from tip, with three minute powdery patches, the largest measuring 1 × 1 mm. All the patches were near the edge of the leaf
Apr. 28	2	14·4	6	All healthy
May 11	2	30·0	9	All healthy. One new shoot, 6 cm., four pairs leaves, all healthy*
Apr. 28	3(*a*)	6·5	3	All healthy
May 11	3(*a*)	11·5	5	,,
Apr. 28	3(*b*)	2·5	1	,,
May 11	3(*b*)	5·5	3	,,
Apr. 28	4	13·5	6	,,
May 11	4	18·0	8	One very minute powdery patch at margin of one leaf at the 4th node from tip†
Apr. 28	5	9·0	5	All healthy
May 11	5	10·0	6	One minute powdery patch on a leaf at 2nd node from tip

* This plant was used in Exp. 5. (See p. 271.)

† This plant was used in Exp. 1. (See p. 265.)

[1] The term "powdery" patch is used to denote the appearance given by densely clustered conidiophores which have produced a powder-like mass of conidia.

(*b*) The material consisted of five pots of cuttings of the Golden Hop taken in the previous autumn from a plant (Ref. No. 288) growing in the Experimental Hop Garden at Wye College. Examination of the plants on April 28th and on May 11th gave the results shown in Table I.

The powdery patches did not increase appreciably in size, and died away in the course of a few weeks. All the plants grew normally and produced by early summer vigorous stems several feet in length; the leaves of these, although exposed repeatedly to inoculation in the greenhouse, showed the complete immunity which had been observed in previous seasons.

Inoculation Experiments with the Golden Hop. In three experiments, leaves were inoculated[1] with conidia from various sources.

Exp. 1. May 5th. One of the plants (pot 4) noted above, was used; two leaves (just expanded) at the 3rd node from the tip were inoculated with conidia, each at three marked places—one leaf, *a*, from the mildew growing on a very susceptible seedling hop (Ref. No. B 18) and the other, *b*, from the mildew on another susceptible seedling of unknown origin. On May 13th leaf *a* showed a very minute cluster of conidiophores at one place of inoculation. No further growth of the fungus took place on this leaf. On May 17th leaf *b* showed at one of the marked places a very minute powdery patch and also, near by, a cluster of five conidiophores. On May 20th another very minute powdery patch had appeared near the margin of the leaf. By May 31st the older patch had died away. By June 16th all the mildew patches had died away on the inoculated leaves, and the 22 leaves produced by this date above the inoculated leaves were all healthy. The plant was then placed in a greenhouse among plants covered with mildew where it was exposed continually to infection; it remained, however, persistently immune.

Exp. 2. May 12th. One of the plants (pot 3) noted above was used; one young leaf at the 2nd node from the tip was inoculated with conidia of the mildew growing on the seedling hop of unknown origin referred to above. Conidia from the same source were also placed at the same time on a similarly situated leaf of the very susceptible seedling B 18. The "control" leaf at the same node, of each plant, was moistened with water. Nine days after inoculation, the leaf of B 18 was fully infected, while no infection occurred on the Golden Hop. The same results were visible on the 21st day. The two "control" leaves remained free from mildew.

Exp. 3. May 17th. One of the plants (pot 2) noted above was used. One leaf at the 3rd and 4th nodes were inoculated with conidia from the seedling hop of unknown origin. Conidia from the same source were also placed at the same time on two similarly situated leaves[2] of a cutting of a "wild" hop which was growing in a pot. The four opposite "control" leaves were treated with water. By May 25th the leaves of the "wild hop" were fully infected, being covered with mycelial growth

[1] The method of inoculation consisted in moistening the surface of the leaf with distilled water and placing conidia therein. In a short time the water evaporated and left the conidia deposited on the leaf.

[2] Both the inoculated leaves at the 3rd node were only partly expanded.

bearing densely clustered conidiophores; the control leaves were free from mildew No infection resulted on the inoculated leaves of the Golden Hop. Very surprisingly, however, on May 31st a few minute clusters of conidiophores, the result of infection from some unknown source, were apparent at the edge of one leaf at the 5th node, and also towards the middle of the control leaf at the 4th node. By June 16th all the patches had died away. No infection took place otherwise on the 16 leaves produced above the inoculated leaves.

In a series of experiments, the details of which are given in a paper published in the present number of the *Annals* (see below, p. 280) scions of the Golden Hop, grafted on a "wild hop" stock, were inoculated with conidia. In two experiments very slight infection resulted, with the production of a few conidiophores; in six the leaves proved to be immune. The dates of the inoculations in the first two experiments were April 29th and 30th; of the cases where negative results were obtained, May 5th, 10th, 19th, 25th. The grafted shoots of the Golden Hop grew vigorously and by early summer had reached a length of several feet; the plants, after resisting inoculation by hand, were placed in a greenhouse and exposed to continual infection for several weeks, and all remained persistently immune.

It is clear from the facts given above that there was no general breaking down of the immunity of the Golden Hop throughout the growing season in 1926, but only a manifestation of a slight and temporary susceptibility at a certain period.

Variety 2. The Seedling Hop, Ref. No. X 91.

Origin. This seedling was raised at Wye College in 1917, the female parent being the Golden Hop and the other an unknown English male hop.

Evidence as to Immunity. Two cuttings, taken in 1919, were grown in pots in 1920 in a greenhouse and remained immune although exposed to continual infection throughout the growing season.

Partial breakdown of Immunity in 1926. The material on which observations were made consisted of 13 cuttings in pots, taken in 1925 from three clone plants (Ref. Nos. OG 9, OH 3, OI 34) derived from the original plant of X 91 in the Experimental Hop Garden at Wye. These plants, stationed in a cold greenhouse, were examined on April 28th and May 11th with the results shown in Table II.

The powdery patches did not increase appreciably in size after May 11th, and very gradually died away. The conidia produced did not apparently cause further infections of the plants. During May and June the shoots of all the plants grew to several feet in length and although

Table II.

Date of exam.	Plant	Length of shoot (cm.)	No. of pairs of leaves	Condition
Apr. 28	OG 9 (1)	3·5	4	One leaf with two minute powdery patches
May 11		8·5	6	Five leaves with small powdery patches, two densely powdery, 4 × 2 and 2 × 2 mm.
Apr. 28	„ (2)	1·0	3	Healthy
May 11		8·5	3	One leaf with powdery patch; lateral shoot with two pairs of leaves, young mildew patch on two leaves
Apr. 28	„ (3)	4·5	5	Three leaves with minute powdery patches
May 11		5·5	6	As on Apr. 28
Apr. 28	OH 3 (1)	3·0	2	Healthy
May 11		8·0	3	Three leaves with weak powdery patches
Apr. 28	„ (2)	5·0	3	Two leaves with powdery patches, one measuring 4 × 3 mm.
May 11		10·5	5	Five leaves with powdery patches up to 4 mm. diam.; powdery patch on stem 4 mm. long
Apr. 28	„ (3) (*a*)	8·0	4	Two leaves with powdery patches
May 11		22·0	7	Five leaves with powdery patches
Apr. 28	„ (*b*)	7·0	4	Two leaves with powdery patches, one measuring 5 × 4 mm.
May 11		13·0	6	Four leaves with powdery patches, including one with eight patches, 2 × 2 mm. or less
Apr. 28	„ (4)	3·5	2	One leaf with minute powdery patch
May 11		10·0	4	Four leaves with small powdery patches
Apr. 28	„ (5)	6·0	3	Healthy
May 11		12·0	5	Two leaves with small powdery patches
Apr. 28	„ (6) (*a*)	4·5	2	Healthy
May 11		16·0	4	Four leaves with small powdery patches
Apr. 28	(*b*)	4·5	1	Healthy
May 11		12·5	3	Two leaves with small powdery patches
Apr. 28	„ (7)	6·5	2	Healthy
May 11		27·0	4	Six leaves with small powdery patches; one leaf at lowest node with 17 minute powdery patches
Apr. 28	OI 34 (1)	8·5	3	One leaf with two powdery patches; stem below 1st pair of leaves encircled by powdery patch 2 mm. long
May 11		27·0	6	Four leaves with powdery patches; patch on stem still powdery; powdery patch on petiole of one leaf
Apr. 28	„ (2)	1·0	3	Healthy
May 11		2·5	4	One leaf with powdery patch, 2 mm. diam.
Apr. 28	„ (3)	1·5	2	Healthy
May 11		3·0	3	One leaf with young mycelial patch

surrounded by mildewed plants in the greenhouse under conditions which ensured frequent inoculation by conidia, they all remained persistently immune. Here again, there appeared to be only a strictly limited period of susceptibility during which infection could take place.

Table III.

Date of exam.	Plant	Length of shoot (cm.)	No. of pairs of leaves	Condition
Apr. 28	310 (1) (*a*)	7·0	3	One leaf with minute powdery patch
May 11		9·0	4	Two leaves, each with minute powdery patches
Apr. 28	(*b*)	5·0	3	One leaf with minute powdery patch
May 11		7·0	4	One leaf with three minute powdery patches at margin
Apr. 28	,, (2)	4·0	4	Healthy
May 11		4·5	4	,,
Apr. 28	,, (3) (*a*)	9·0	5	,,
May 11		14·0	6	One leaf with two minute, scarcely powdery, patches
Apr. 28	(*b*)	8·0	4	Healthy
May 11		12·0	6	,,
Apr. 28	,, (4)	6·0	3	,,
May 11		10·0	6	,,
Apr. 28	,, (5)	10·0	4	One leaf with very minute powdery patch
May 11		10·5	5	Leaves healthy; minute powdery patch dying away
Apr. 28	,, (6) (*a*)	8·0	3	Healthy
May 11		19·0	4	One leaf with very minute powdery patch at margin
May 11	(*b*)	3·5	2	Healthy
Apr. 28	OJ 12 (1)	6·0	4	,,
May 11		8·0	5	,,
Apr. 28	,, (2) (*a*)	14·0	3	,,
May 11		24·0	5	Two leaves each with three slightly powdery patches
Apr. 28	(*b*)	10·0	2	Healthy
May 11		19·0	3	One leaf with weak powdery patch
Apr. 28	(*c*)	4·0	2	Healthy
May 11		7·0	3	,,
Apr. 28	,, (3) (*a*)	7·0	4	,,
May 11		9·5	5	,,
Apr. 28	(*b*)	3·0	2	,,
May 11		5·0	4	,,
Apr. 28	,, (4)	9·0	5	,,
May 11		11·0	6	,,
Apr. 28	,, (5)	6·5	3	,,
May 11		8·0	5	,,
Apr. 28	,, (6)	10·5	4	,,
May 11		29·0	7	One leaf (at the 2nd node from the base) with a yellowish area where a weak growth of scattered conidiophores occurred*

* The term "sub-infection" has been used to denote cases in which inoculation is followed by the production merely of a few scattered conidiophores. See (7) (8).

Variety 3. The Seedling Hop, Ref. No. 310.

Origin. This is the same as that given above for the variety X 91.

Evidence as to Immunity. Four cuttings taken in 1919 were grown in pots in 1920 in a greenhouse where they remained immune, although continually exposed throughout the growing season to inoculation by conidia from surrounding mildewed plants.

Partial breakdown of Immunity in 1926. The material on which observations were made consisted of (1) six cuttings in pots taken in the autumn of 1925 from the original plant of Ref. No. 310, and six cuttings in pots of the "clone plant," Ref. No. OJ 12, both growing in the Experimental Hop Garden at Wye College; and (2) eight rooted plants raised at East Malling during 1925 from cuttings taken from the clone plants of Ref. No. 310 growing at the Research Station, East Malling, and transferred to pots at Wye during the winter of 1925. Both (1) and (2) were kept in an unheated greenhouse at Wye during the winter of 1925–6 and the spring and summer of 1926.

(1) The examination of these plants on April 28th and May 11th gave the results shown in Table III.

(2) These plants were examined only on April 28th, with the following results:

Table IV.

Plant	Length of shoot (cm.)	No. of pairs of leaves	Condition
310 (1) (*a*)	55·0	12	One leaf (at 7th node from tip) with very minute powdery patch
(*b*)	15·0	6	One leaf (at 3rd node from tip) with large powdery patch
„ (2)	90·0	—	All leaves healthy*
„ (3)	90·0	—	Large, very powdery patch (4 × 4 mm.) on one leaf (5th node)
„ (4)	80·0	—	Powdery patches on two leaves (4th node)
„ (5) (*a*)	100·0	—	All leaves healthy
(*b*)	60·0	—	„
„ (6) (*a*)	60·0	—	„
(*b*)	20·0	—	„
„ (7) (*a*)	60·0	—	„
(*b*)	30·0	—	„
„ (8)	30·0	—	Two leaves (at 2nd node from the base) each with a small powdery patch

* The plant was used in Exp. 7. (See p. 272.)

The plants of (1) and (2) were kept through May and June in a greenhouse where they were exposed constantly to infection by conidia from contiguous plants covered with the mildew. No further infections

resulted. Here again, for the third time, the phenomenon of susceptibility was only temporary.

In one set of grafting experiments (see below, p. 281) scions of the variety 310, grafted on two different stocks, were inoculated, on May 28th and June 8th, with conidia taken from the very susceptible seedling hop, Ref. No. B 18. No infection resulted. In another set of grafting experiments, the variety 310 was used as the stock with grafts of a "wild hop." Inoculations on May 18th, June 3rd and 11th, with conidia taken from B 18 resulted in full infection of the grafts, while the leaves of 310 proved completely immune. The grafted plants were placed in the greenhouse where they were exposed to constant inoculation during the summer, but no infection resulted on the stocks or scions of the variety 310.

Two hypotheses may be advanced to account for the presence of the mildew on these "immune" plants: (1) the evolution of one or more specialised forms of *S. Humuli* able to attack "immune" varieties; (2) the effect of abnormal growing conditions either on (*a*) the host plant, so that its cells temporarily lost the power of preventing the fungus from infecting them, or on (*b*) the fungus, so that its spores temporarily acquired an increased power of infection.

On *a priori* grounds, the first hypothesis has much to support it. The phenomenon of specialisation of parasitism is found to a high degree in the Erysiphaceae. For example, in the species *Erysiphe Graminis*, the specialisation has proceeded to such an extent that we find (8) not one, but several specialised forms with distinctive powers of infection towards different species of the host genus *Bromus*. Experiments have shown (6) that the form of *S. Humuli* on species of *Humulus* is specialised to this genus. Further, Steiner (10) has shown that a number of specialised forms of *S. Humuli* exist on *Alchemilla* with distinctive powers of infection towards different species. It would not be unreasonable to suppose that, similarly, several specialised forms may evolve from the form on the genus *Humulus*, able to attack hitherto immune varieties. The circumstances may be conceived as being favourable for this. The immune plants were surrounded by susceptible plants heavily infected with the mildew. If among the millions of conidia being produced on these, one conidium varied in the direction of being able to infect an immune plant, and if then the conidia produced on this plant showed the same infective powers, a new specialised form would have arisen. In the Uredineae, the careful work of Stakman and his collaborators has shown (11) that specialisation of parasitism has taken place to a

remarkable degree; the specialised form *Tritici* of *Puccinia Graminis* has been found to be composed of a large number of further specialised forms with distinctive powers of infection towards different varieties of wheat.

With regard to the occurrence of mildew on the "immune" varieties of hops described above, evidence was sought as to whether a new specialised form might be evolving.

A few experiments were able to be made in which the infective powers of conidia produced on an "immune" variety were compared with those of conidia taken from a susceptible variety.

Inoculation experiments with conidia taken from "immune" varieties of hops.

Exp. 4. April 29th. Conidia were taken from the powdery patches produced on the plants OH 3 (2) and (3) of the variety X 91 (see above, Table II), and deposited on the two leaves (not quite fully expanded) of the 2nd node (from the tip) of a shoot 52 cm. long of a plant of the Golden Hop which for several years past had been grown in a pot in the greenhouse and had proved immune. On May 10th one of the inoculated leaves showed a number of scattered groups of conidiophores over the inoculated area; on the other inoculated leaf a very few scattered or more or less clustered conidiophores occurred at the place of inoculation—in both cases the appearance was that of "sub-infection" only. As, however, at this date the leaves at the next lowest node showed similar minute spots of mildew, but rather more powdery, the evidence as to the origin of the mildew on the inoculated leaves must remain doubtful. By May 31st, the little patches on the inoculated leaves were still evident but had not perceptibly increased in size; no mildew appeared on the leaves of the nodes higher up. On June 16th, the powdery patches began to die away, exposing to view minute raised brown patches of leaf cells. Such "injury spots" are characteristic of attacks by the mildew on the leaves of "semi-immune" plants (see (3), p. 257). No mildew occurred on the 22 leaves eventually produced above the inoculated leaves. If the mildew produced in this experiment on the inoculated leaves be considered as the result of the inoculation, this would be no evidence that the conidia produced on X 91 had any exceptional powers of infection, since weak infection of the Golden Hop can sometimes be produced by conidia taken from fully susceptible plants (B 18 and an unlabelled seedling) (see above, p. 265).

Exp. 5. May 7th. One leaf at the 2nd node from the tip of a shoot of the Golden Hop[1] was inoculated with conidia taken from "immune" plants of X 91 (OI 34, pot 1; OH 3, pots 2 and 3 (see Table II, p. 267)) and the other leaf at the same node with conidia from the fully susceptible variety B 18. Each leaf was inoculated in a drop of water at three marked places and when the water had dried, the deposited mass of spores was plainly visible. No infection resulted on either leaf. At the time of inoculation all the leaves of this plant of the Golden Hop were believed to be free from mildew; on May 17th, three very minute powdery patches were visible on one leaf near the base of the shoot (possibly overlooked on May 11th); these patches

[1] This plant was pot 2, in Table I (p. 264).

had increased slightly in size by the 21st, but no spread of the mildew took place and by the 31st, they had died away.

Exp. 6. May 25th. Two leaves (one-quarter expanded) at the 2nd node from the tip of shoots of two plants (pots 2 and 4) of the immune variety OH 3 (= X 91) were inoculated, one leaf with conidia taken from OH 3, pot 5 (see Table II) and the other leaf with conidia from a plant of the susceptible variety B 18. Water only was placed on the control leaves at the same node. No infection resulted in either case.

Exp. 7. April 30th. Two leaves at the 3rd node from the tip of a shoot of the rooted set (pot 2) of the immune variety 310 (see Table IV) were inoculated, one leaf at a marked place near the base of the centre lobe, with conidia produced on the leaves of another rooted set (pot 1) of 310 (see Table IV) and the other leaf, at three marked places (one on each lobe) with conidia taken from B 18. No infection resulted on either leaf.

Exp. 8. May 17th. One leaf (partly expanded) at the 2nd node from the tip of a rooted set (pot 6) of the variety 310 (see Table IV) and one leaf at the 2nd node of a plant of B 18, were heavily inoculated with conidia taken from the "immune" variety X 91 (OH 3, pots 2, 3, 4, 5, 7; see Table II). The opposite leaf at the node was treated with water only. By May 25th the leaf of B 18 was fully infected and by June 2nd, bore patches of densely clustered conidiophores "powdery" with accumulated conidia; the control leaf remained healthy. The inoculated leaf of 310 remained uninfected; the places where the conidia had been deposited were marked by little areas of dead brown epidermal cells—the typical reaction shown by the leaf of many varieties of hops immune from mildew.

It will be seen that the experiments detailed above give no evidence that the conidia produced on an "immune" plant possess distinctive powers of infection indicative of the evolution of a new specialised form. In this respect, the results obtained in Exp. 8 seem conclusive. It is an interesting fact that a conidium produced on an "immune" plant may be unable to infect its host[1], and one that suggests that the plant in question must have passed from a susceptible to an immune state.

The supposition, then, that the mildew on an "immune" variety might denote the advent of a new specialised form, finds no support from the above facts. Further evidence that these "immune" plants were not serving as the birth-place of new specialised forms is found in the fact that, as mentioned earlier, the mildew died away on all of them after a few weeks, without spreading to fresh leaves, under conditions in which the mildew attacked virulently the new leaves produced on ordinarily susceptible plants.

[1] We may compare the results of these experiments with those obtained where a plant has been artificially rendered susceptible to the attacks of the "wrong" specialised form of the mildew by the use of heat, alcohol, ether and other agents (see (9)). Conidia produced on such plants are not able to infect an untreated plant.

Nevertheless one may speculate whether in such cases as the above, where a biologic form of the mildew is able to live for a time on a host plant which normally is completely resistant, may not represent the first step in the evolution of a further biologic form adapted to certain members of the host genus. Possibly under a continuance of those special circumstances (which were probably weather conditions) which would enable the mildew to exist for a longer time on the new host plants, the conidia produced on them would possess the power of infecting the host plant under normal weather conditions. Of that, however, we have as yet no evidence.

We will now consider the second hypothesis, viz. whether there is any evidence for attributing the temporary susceptibility shown by the immune plants to abnormal growing (weather) conditions, the actual existence of which is indicated by the figures given below. The days when the first occurrence of mildew on immune plants was noted (April 28th) were dark and cold and the growth of the plants in the greenhouse either stopped entirely or there was only a very slight elongation of the stems. Unfortunately, figures of the temperatures in the greenhouse are not available. Mr A. H. Bird, Meteorological Observer at Wye College in connection with the Ministry's Agricultural Meteorological Scheme, has kindly supplied me with the chart showing the temperature of the air in the open at Wye for April and May, 1926. It

Temperature of the Air. Mean of Daily Values (° F.).

1926 Week ending	England. South-Eastern Counties				Wye, Kent			
	Max.	Min.	Mean (adjusted)	Deviation from normal*	Max.	Min.	Mean (adjusted)	Deviation from normal for S.E. England*
Apr. 3	57·6	39·0	48·0	+4·0	59·7	35·5	47·0	+3·0
„ 10	57·8	43·5	50·3	+4·7	58·0	44·6	51·0	+5·4
„ 17	55·8	39·8	47·4	+1·5	57·0	35·9	45·9	0
„ 24	52·5	39·2	45·5	−1·4	51·6	37·7	44·3	−2·6
May 1	55·1	45·5	50·1	+1·6	55·3	44·3	49·4	+0·9
„ 8	54·4	40·8	47·2	−2·2	51·7	41·1	46·1	−3·3
„ 15	54·8	41·9	48·0	−3·1	54·1	41·1	47·2	−3·9
„ 22	58·5	42·9	50·2	−2·5	58·0	41·3	49·1	−3·6
„ 29	65·3	51·0	57·8	+3·9	67·6	49·6	58·0	+4·1
June 5	60·9	49·0	54·5	−1·3	61·0	48·0	54·1	−1·7
„ 12	63·2	50·6	56·4	−0·5	61·7	49·3	55·1	−1·8

* The "normal" is the mean for a 35 year period, and as regards Wye, is non-existent. The figures are not shown in the above table, but are arrived at by adding or subtracting the figures in the "deviation" column to or from those in the "adjusted mean" column.

will be observed that in the week ending April 24th, the minus deviation from the normal was 2·6 degrees, and those for the weeks ending May 8th, 15th and 22nd were all over 3 degrees. There does seem to be some evidence, then, that one factor influencing the temporary loss of immunity noted in 1926 may have been abnormal growing conditions. Other evidence exists, however, with regard to cases of loss of immunity noted in previous seasons, which must be considered here. In 1917, the seedling variety OR 38, which previously had shown in the greenhouse complete resistance to the mildew, exhibited slight and temporary susceptibility. This phenomenon was observed twice. In the first instance an inoculated leaf became weakly infected. The weather conditions were abnormal at the time (March, 1917); rapid changes of temperature occurred and the stem of the plant made scarcely any growth. Here it would seem reasonable to attribute the breakdown of immunity to the effect of weather conditions. In another case, however, susceptibility occurred under different circumstances. A leaf of OR 38 was heavily inoculated with conidia, together with leaves of two susceptible varieties; the latter became infected and OR 38 proved immune. On the 16th day after inoculation (May 22nd, 1917) a "control" leaf (at the 5th node from the tip) of the stem of OR 38 bore one minute powdery patch of mildew, the inoculated leaf, as well as all the other leaves on the stem being at this date and subsequently, entirely free from the mildew. Conidia taken from the patch failed to infect a young leaf of OR 38. Further details will be found in the account published in 1919 ((2), pp. 84, 85). The conclusion then reached was that an immune plant in the greenhouse may show strictly local susceptibility without the general immunity being lost, and that this susceptibility was due to unknown causes. A similar and unexplained case of local susceptibility occurred in the open in 1918. A plant of the immune variety OB 34 (see (3), p. 256) growing in the Experimental Hop Garden at Wye College, produced a crop of some hundreds of healthy "hops" (strobiles), except for one young "hop" which alone was smothered with the conidial stage of the mildew.

Such cases as the above[1], incline one to suspect that some other factor besides weather conditions exists which is able to cause localised susceptibility. On the other hand, again, there is the case already recorded, of a group composed of a number of different seedling varieties of hops which when grown in the greenhouse, remain season after season

[1] There is also the case of the Golden Hop in 1917, where as already mentioned above (p. 263) abnormal weather conditions had no effect upon its immunity.

completely resistant to the mildew but which, when grown in the open, sometimes prove at the end of the summer, to be susceptible, occasionally even to the highest degree[1]. A detailed history of the varieties of this group has already been given[2]. Here, again, it appears that the susceptibility is induced by special weather conditions and not by new specialised forms of the fungus.

Summary.

1. Three varieties of hops which had previously shown complete resistance to the attacks of *S. Humuli*, became slightly infected in the greenhouse in the spring of 1926.

2. The susceptibility shown was only temporary; all the infected plants during May and June again acquired their power of complete resistance.

3. The conidia produced on an "immune" plant proved unable to infect that plant, although able to infect a susceptible variety.

4. It appears probable that abnormal weather conditions (possibly low temperature) brought about the temporary susceptibility.

BIBLIOGRAPHY.

(1) Salmon, E. S. (1917). On Forms of the Hop (*H. Lupulus* L.) resistant to Mildew (*Sphaerotheca Humuli* (DC.) Burr.). *Journ. Agric. Sci.* viii, 455.

(2) —— (1919). *Idem* II. *Journ. of Genetics*, viii, 83.

(3) —— (1919). *Idem* III. *Ann. App. Biol.* v, 252.

(4) —— (1920). *Idem* IV. *Ibid.* vi, 293.

(5) —— (1921). *Idem* V. *Ibid.* viii, 146.

(6) —— (1907). Notes on the Hop Mildew (*S. Humuli* (DC.) Burr.). *Journ. Agric. Science*, ii, 330.

(7) —— (1904). On Specialisation of Parasitism in the *Erysiphaceae*. II. *The New Phytologist*, iii, 111.

(8) —— (1904). On *Erysiphe Graminis* and its adaptive parasitism within the genus *Bromus*. *Annal. Mycolog.* ii, 255.

(9) —— (1905). Further cultural experiments with "Biologic Forms" of the *Erysiphaceae*. *Annals of Botany*, xix, 125.

(10) Steiner, J. A. (1908). Die Spezialisation der *Alchemillenbewohnenden Sphaerotheca Humuli* (DC.) Burr. *Centralbl. f. Bakt.* etc. xxi, 677.

(11) Stakman, E. C. and Levine, M. N. (1922). The determination of biologic forms of *Puccinia Graminis* on *Triticum* spp. *Minn. Agr. Exper. Station Tech. Bull.* 8.

[1] In the case of one of these seedlings, Z 25, a few small patches of mildew appeared in 1919 on a few of the young leaves on Aug. 7th after a spell of abnormally cold, dull weather; these patches soon died away and in October the plant was entirely free from mildew. (See (5), p. 152.)

[2] (1), p. 457; (2), pp. 84, 85, 87; (3), pp. 254–8; (4), pp. 298, 306; (5), pp. 150–158.

(*Received February 26th*, 1927.)

GRAFTING EXPERIMENTS WITH VARIETIES OF HOPS RESISTANT TO THE HOP POWDERY MILDEW, *SPHAEROTHECA HUMULI* (DC.) BURR.

By Prof. E. S. SALMON and W. M. WARE, M.Sc.
(*Mycological Department, South-Eastern Agricultural College, Wye, Kent.*)

(With Plate XVIII.)

The experiments on immunity described below, in that they have as an essential feature the employment of grafting, are an attempt to further the work of Roach(1) who has so successfully used this method for the investigation of the problem of potato immunity from wart disease (*Synchytrium endobioticum*). This author has demonstrated that when varieties of potatoes, resistant or susceptible to the attacks of *Synchytrium endobioticum*, are used as stocks and grafted with scions of varieties showing the opposite reaction to this fungus, the tubers produced are not influenced as regards their behaviour to the parasite.

Under the stimulus of the announcement of the above discovery, we were led to experiment firstly with the grafting of the hop plant and, secondly, with the effect of grafting on the immunity or susceptibility of the scion to the attacks of the hop mildew, *Sphaerotheca Humuli* (DC.) Burr. In 1925 we showed(2) that, using a method similar to that employed by Roach, the actual process of grafting the hop plant was not difficult. In the present paper the results are given of inoculation experiments made with conidia of the hop mildew, *S. Humuli*, on immune and susceptible scions respectively.

The only alteration in technique from that previously described(2), in the process of grafting employed in these experiments consisted in the use of a trace of rubber solution to secure the last fold of the thin india-rubber tape which was the only binding material around the graft. The cleft method of grafting alone was used and the stock was cut at about 2 inches from the ground. No elongation of the stock was ever noticed to take place and the growth of the scion was therefore measured from the ground level. (See Tables I and II.)

There were two ways in which the relation of the composite plant to the mildew might be investigated: by grafting (1) susceptible scions on immune stocks, and (2) immune scions on susceptible stocks.

(1) In the first case any substance present in the stock, concerned in conferring immunity, might, if translocatable, be expected to influence the susceptibility of the scion; or again, if susceptibility be due to the presence of some substance found in susceptible stocks but absent from the immune stocks, then the cessation of supply might induce some degree of immunity.

(2) The same two considerations apply equally, *mutato mutando*, when immune scions are grafted on susceptible stocks. Both of the above types of graft were made.

After having been grafted, the whole plant or sometimes only the shoot concerned, was kept covered by a bell-jar or plugged lamp-glass for ten days. The grafted plants were placed in a shaded part of the greenhouse, out of direct sunlight, for the whole of that period and for a day or two after the glass covering had been removed. The rubber binding was then pulled away. At the time of grafting or within the following month, every internode of the grafted shoots was measured and the state of development of the leaves noted. In this way it was possible to determine, before selecting the plant for inoculation, not only whether growth had taken place, but also which pairs of leaves had unfolded from the bud during a part of the time that the scion had been receiving nourishment from the stock. Infection tests were only made upon leaves which had developed at some time subsequent to the date of the first of these records; any leaves which had then even been distinguishable (*i.e.* with tips projecting from the terminal bud) were neglected for this purpose. In most cases very considerable growth took place before any infection experiments were made and often the leaves used for inoculation were situated at a distance of four or five new internodes above the position occupied by the terminal bud at the time when the preceding record was made.

The varieties of hops used in the experiments, were as follows:

Class 1. Immune Varieties.

1–3. *"Golden Hop"; seedlings Ref. Nos. X* 91 *and* 310. The facts concerning the origin and immunity of these three female varieties have already been given in a paper by one of us (see above, p. 264). Under normal circumstances, these three varieties are completely resistant to the attacks of *S. Humuli*.

4–5. *Ref. Nos. II* 124 *and V* 93. These two male varieties were raised in 1913 from seed of a wild plant or plants of *Humulus Lupulus* obtained from Italy. As has been pointed out (3), both these varieties, when grown in the greenhouse, are completely resistant to the attacks of *S. Humuli*, although No. V 93, when grown in the open, may show a slight degree of susceptibility in the late autumn.

6. *Unnamed Immune.* This plant was one of a number of immune seedlings raised from the seed of wild *H. Lupulus* obtained from Italy, which had shown in the greenhouse for a number of seasons complete resistance to *S. Humuli*. Owing to a label in the pot having been lost, its further identification could not be established[1].

Class 2. Susceptible Varieties.

1–3. "*Wild Hop,*" *A, B and C.* These were cuttings taken from (uncultivated) hops found growing wild in the hedge in three different localities at Wye[2].

4. *Tutsham.* This is a commercial variety commonly cultivated in Kent; its degree of susceptibility to *S. Humuli* is unknown.

5. *Ref. No. B* 18. This is a variety of unknown parentage; it has been named the "Foundling" and has been described in the *Journal of the Board of Agriculture*, May, 1915. It has proved extremely susceptible in the greenhouse; occasionally its shoots, soon after their appearance above ground, become completely mildewed all over their surface and then hypertrophied.

Method of Inoculation.

The powdery mildew of the hop (*S. Humuli* (DC.) Burr.) forms on the surface of the leaves (and occasionally on the stems) of susceptible varieties of hops, small or large mycelial patches on which densely-clustered conidiophores, bearing chains of conidia, are produced. Under favourable conditions for growth and in still air, the patches become "powdery" or "mealy" from the accumulation of ripe, detached conidia which have been produced successively in the greatest profusion.

In the experiments described below, the method of inoculation was as follows, unless otherwise stated. The leaf to be inoculated was moistened on the upper surface with distilled water. Using a small, clean camel-hair brush, conidia were placed in the film of water; the water evaporated in a short time and left the conidia deposited on the

[1] Very possibly the plant was Ref. No. OB 34. (See (3), p. 153.)

[2] In the South of England, such plants are believed to be not truly wild but to originate from cuttings of commercial varieties.

epidermis. The plants were in a greenhouse by themselves and were not covered over; the uninoculated leaf at the same node, or the remaining leaves on the plant, served as controls.

Except in the case of Exp. 1 *a* (Table I) the conidia used for inoculation were taken from clone plants of the variety B 18. As the possibility of the existence of a specialisation of parasitism was being explored (see above, p. 271), notes were kept of the exact source of the conidia used in each experiment but as no evidence was obtained that the infective powers of the conidia produced on different clone plants of B 18 varied in any way, these details are not given here. Seventy-six leaves of susceptible varieties (scion or stock) were inoculated and 72, *i.e.* 94·7 per cent., became infected.

Results of Experiments.

Class 1. *Scions of Immune Varieties grafted on Susceptible Stocks.*

Scions of six different immune varieties were grafted on stocks of four different susceptible varieties. The details of the grafting and the results of the inoculations are given in Table I and further particulars relating to the growth of the mildew in each experiment are given on p. 280. It will be seen that with the exception of the Golden Hop, where "sub-infection" occurred in two experiments, the immune scions remained immune. A typical case may be taken as illustration. In Exp. 7 *a* (see Table I) a scion of the immune variety V 93 was grafted, on March 12th, on the susceptible stock B 18. By March 29th the scion measured (from the ground) 15·6 cm. and possessed four pairs of leaves above the graft. On May 5 inoculations were made on leaves at the 8th and 9th nodes above the graft, the scion now reaching 79·0 cm., or more than five times its original height. The scion completely resisted infection. The same scion was again used on May 21st, by which time it had grown to 115 cm., more than seven times its original height; leaves at the 12th and 13th nodes above the graft were inoculated and proved to be completely resistant. In Exp. 11 the same scion was inoculated on three occasions on leaves at the 5th, 6th, 8th, 9th, 14th and 15th nodes above the graft, the scion being finally more than twelve times its original height; on each occasion the scion proved to be immune. In the case of the Golden Hop, the "sub-infection" which resulted in May on the four inoculated leaves in Exps. Nos. 1 *a* and 2 *a* was exactly similar to that which had been observed on ungrafted plants during April and May, 1926, and cannot therefore be ascribed to the influence of the susceptible stock. It will be noted that each scion was again inoculated after fresh growth had been made (Exps. Nos. 1 *b* and 2 *b*), and that they proved completely resistant.

Class 2. *Scions of Susceptible Varieties grafted on Immune Stocks.*

Scions of four different susceptible varieties were grafted on stocks of five different immune varieties. The results of the inoculations given in Table II and the particulars of the experiments given on p. 283 show that all the scions retained their original degree of susceptibility.

Details of Grafting Experiments.

1. *Immune Scions on Susceptible Stocks.* (See Table I.)

Exp. 1 *a*. Two leaves at the 3rd[1] node of the scion (Golden Hop) were inoculated, one with conidia taken from a plant of B 18, and the other with conidia from an unnamed seedling hop. The leaves were very young and only partly expanded; the conidia were placed in drops of distilled water on each of the three lobes of the leaf. Four leaves, at the 3rd and 4th nodes of the stock[2] (Wild Hop C), were similarly inoculated. On the 11th day all the four leaves of the stock were infected and bore mycelial patches with conidiophores; no infection was visible on the scion. By the 18th day the leaves at the 3rd node of the stock were almost continuously covered with mycelial growth, bearing dense powdery clusters of conidiophores; those at the 4th node were fully but less virulently infected. Of the inoculated leaves of the scion, one bore 2 conidiophores on its left lobe[3], about 12 more or less isolated conidiophores and a cluster of 6 on the middle lobe, and a single conidiophore on the right lobe. The other leaf bore two minute clusters of 2 and 4 conidiophores, respectively, on the middle lobe. No mycelial growth was evident, and the general appearance was similar to that of "sub-infection[4]." No infection occurred elsewhere on the leaves of the scion.

Exp. 1 *b*. The above scion was used again 11 days later, by which time it had made a fresh growth of 16 cm. Two leaves at the 2nd and 3rd nodes of the scion, and similarly of the stock, were inoculated. By the 7th day there was abundant mycelial growth on the two inoculated leaves of the stock, and by the 11th day dense powdery masses of conidiophores; the control leaves at the same nodes were free from mildew. No trace of infection occurred on the two leaves, or others, of the scion.

Exp. 2 *a*. In the case of both scion (Golden Hop) and stock (Wild Hop C), four leaves at the 2nd and 3rd nodes were inoculated, the conidia being placed in a drop of water on each of the three lobes of the leaf. By the 10th day all the leaves of the stock were fully infected, and clusters of conidiophores were beginning to be formed. No signs of infection were apparent on the scion. By the 17th day the inoculated leaves of the stock were almost continuously covered with mycelial growth bearing powdery clusters of conidiophores.

Of the inoculated leaves of the scion, one at the 3rd node bore at two places on its right lobe minute clusters of 5–7 conidiophores; on its middle lobe, a few solitary conidiophores, while no infection occurred on the left lobe. As regards the other leaf at the 3rd node, a very few solitary conidiophores appeared on the right and middle lobes and no infection on the left lobe. At the 2nd node of the scion, one

[1] *I.e.* counting from the tip of the shoot.

[2] In all cases, the inoculated leaves of the stock were not on the grafted stem but on a shoot coming from the same plant, since it was necessary to secure leaves of the same age and condition. Owing to their unsuitability in this respect, the leaves of the stock below the graft were never inoculated.

[3] The dorsal surface of the leaf was viewed with the petiole above.

[4] The term "sub-infection" has been used to denote the production of a few scattered conidiophores.

leaf showed 3 weak conidiophores on the left lobe and the other leaf a group of 5 conidiophores on the right lobe, 8 scattered conidiophores on the middle lobe and 3 isolated conidiophores on the left lobe. No infection occurred elsewhere.

Exp. 2 *b*. The above graft was used 19 days later when a fresh growth of 49·8 cm. had been made. One leaf at the 2nd and 3rd nodes of both the stock and scion were inoculated, the control leaf opposite being treated with water only. Full infection resulted on the stock, the control leaves remaining free; no trace of infection was observable on the scion.

Exp. 3 *a*. Four leaves at the 3rd and 4th nodes of the scion (Golden Hop) were inoculated, and two leaves at the 3rd node of a long stem of the stock (Wild Hop C) and two leaves at the 3rd node of a short shoot of the stock (of the same length as that of the scion). All the leaves of the stock became infected, and no infection resulted on the scion.

Exp. 3 *b*. The above scion was used 20 days later, when a fresh growth of 41 cm. had been made. Two leaves at the 2nd and 3rd nodes of the scion and two leaves at the 3rd and 4th nodes of the stock were inoculated, the control leaves being treated with water only. By the 22nd day the leaf at the 3rd node of the stock was somewhat weakly infected; no infection resulted on the leaf at the 4th node. The control leaves remained healthy. No trace of infection resulted on the scion.

Exp. 4 *a*. Two leaves at the 3rd node of both the scion (Golden Hop) and the stock (Wild Hop C) were inoculated. Infection occurred on the stock but not on the scion.

Exp. 5 *a*. Two leaves at the 2nd node of both the scion (No. 310) and the stock (Wild Hop A) were inoculated. The leaves were about one-quarter expanded. By the 7th day the leaves of the stock were fully infected; no infection occurred on the scion, but pale areas were visible at the places where the conidia had been deposited[1].

Exp. 6 *a*. Two leaves at the 2nd node of both the scion (No. 310) and the stock (Tutsham) were inoculated. The leaves were about one-half expanded. By the 22nd day the leaves of the stock were fully infected, while no trace of infection occurred on the scion.

Exp. 7 *a*. Four leaves at the 2nd and 3rd nodes of the scion (No. V 93) were inoculated. No infection resulted. The stock was not inoculated in this experiment.

Exp. 7 *b*. The above scion was used 16 days later, when a fresh growth of 36 cm. had been made. Four leaves at the 2nd and 3rd nodes were inoculated. The leaves at the 2nd node were very small (1·5 to 2·0 cm. long) and at the 3rd node only about one-half expanded. No infection occurred. The stock was not inoculated in this experiment.

Exps. 8 *a and* 9 *a*. Two scions (No. II 24) on B 18 stock were used. Two leaves on an ungrafted shoot of the same stock were also inoculated. By the 16th day full infection had resulted on all three lobes of each of the two leaves of the stock; no infection occurred on the scion.

Exps. 8 *b and* 9 *b*. The two scions were again used 20 days later, by which time fresh growths of 57 cm. and 67 cm. (respectively) had been made. On one scion one leaf at the 2nd node was inoculated, and on the other one leaf at the 3rd. One leaf

[1] The same phenomenon has been observed when conidia have been deposited on the leaves of ungrafted resistant varieties.

at the 3rd node of an ungrafted stem of the stock was also inoculated. All the inoculated leaves were about one-half expanded. The control leaves at the same nodes were treated with water only. By the 7th day complete infection had resulted on the stock, large mycelial patches (up to 0·5 cm. in diam.) bearing densely clustered conidiophores were present on all three lobes of the leaf; the control leaf remained healthy. No infection occurred on the scion; the inoculated leaves showed conspicuous, pale, often somewhat wrinkled areas where the conidia had been deposited.

Exp. 10 *a*. Four leaves at the 2nd and 3rd nodes of the scion (unnamed immune) and four leaves at the same nodes of the stock (No. B 18) were inoculated. By the 11th day the four leaves of the stock were all fully infected; by the 15th practically the whole of the upper surface of each leaf was continuously covered with the mycelial growth bearing conidiophores so dense powdery with accumulated conidia that a mist-like mass of conidia was dispersed through the air on the leaves being touched. No infection occurred on the scion.

Exp. 10 *b*. The same scion was again used 15 days later. Two leaves at the 2nd node were inoculated, as well as two leaves of the stock at the 2nd node. By the 11th day both leaves of the stock were almost continuously covered, on the upper surface, with mycelial growth bearing densely clustered conidiophores, already more or less "powdery" with accumulated conidia. No infection occurred on the scion; both the inoculated leaves showed pale areas where the spores had been deposited.

Exp. 11 *a*. Four leaves at the 2nd and 3rd nodes of the scion (unnamed immune), and four leaves at the same nodes of the stock (No. B 18) were inoculated. The leaves at the 2nd node of both scion and stock were about one-sixth expanded. By the 11th day the leaves of the stock at the 2nd node were fully infected and those at the 3rd node, weakly. By the 15th day the leaves at the 2nd node were so continuously covered with densely massed conidiophores bearing conidia, that a mist-like cloud of conidia dispersed on the leaves being touched; the leaves at the 3rd node bore more scattered but dense masses of conidiophores. No infection occurred on the scion.

Exp. 11 *b*. The scion was used again 16 days later by which time a fresh growth of 33·2 cm. had been made. Two leaves at the 2nd and 3rd nodes of both the scion and stock were inoculated. The leaves at the 2nd node were only partly expanded. The four control leaves at the same nodes were treated with water only. By the 8th day the leaf of the stock at the 2nd node was fully infected and showed over its surface an almost continuous mycelial growth, bearing densely clustered young conidiophores; the leaf at the 3rd node was infected with scattered mycelial patches and clustered young conidiophores. The control leaves remained free. No infection occurred on the scion. The inoculated leaves showed pale, slightly shrunken areas where the conidia had been placed—these were absent from the control leaves.

Exp. 11 *c*. The scion was again used 17 days later, when a fresh growth of 67 cm. had been made. Two leaves at the third node, and one leaf at the 2nd node of both scion and stock were inoculated. In both cases the leaves at the 2nd node were very small (7–8 mm. long); at the 3rd node they were about one-quarter expanded. The control leaf at the 2nd node was treated with water only. By the 13th day all the inoculated leaves of the stock were fully and heavily infected; the control leaf was healthy. No infection of the scion occurred and, as before, the inoculated leaves showed pale areas where the conidia had been deposited.

Exp. 12 *a*. Four leaves at the 2nd and 3rd nodes of both the scion (unnamed immune) and the stock (No. B 18) were inoculated. By the 11th day the four leaves on the stock were fully infected. No infection resulted on the scion.

Exp. 12 *b*. The above scion was again used 15 days later, when a fresh growth of 24·5 cm. had been made. Two leaves at the 2nd node of both the scion and stock were inoculated. By the 6th day the leaves of the stock were fully infected, whereas those of the scion were free.

Exp. 13 *a*. Four leaves at the 2nd and 3rd nodes of the scion (No. II 30) were inoculated. No infection resulted. It was noted that the scion made no growth during the experiment. No inoculation was made of the stock.

2. *Susceptible Scions on Immune Stocks.* (See Table II.)

Exp. 1 *a*. Four leaves of the scion (Tutsham) were inoculated at the 3rd and 4th nodes; those at the 3rd node were only half expanded. Two leaves of the stock at the 2nd node (Golden Hop) were inoculated. The leaves were moistened by spreading distilled water over them and conidia deposited over the damp surface; in the case of the leaves at the 4th node however, owing to the difficulty of getting the water to wet the surface of the older leaves, the conidia were deposited in drops of water. By the 7th day the two leaves at the 3rd node of the scion were seen to be fully infected; by the 13th day these leaves bore crowded and very powdery patches of conidiophores. No infection occurred on the leaves of the scion at the 4th node (6th above graft). This non-infection of the scion may be attributed possibly to the age and condition of the leaf. No infection occurred on the stock.

Exp. 2 *a*. Two half-expanded leaves at the 2nd node of the scion (Wild Hop B) and one leaf at the 2nd and 3rd nodes of the stock (No. 310) were inoculated, water only being placed in the latter case on the control leaf at each node. By the 7th day the two leaves of the scion were fully infected and by the 13th day bore very numerous dense powdery patches of conidiophores. No infection occurred on the stock; pale areas were observed on the epidermis where the conidia had been deposited.

Exp. 2 *b*. The above plant was used again 16 days later, by which time the scion had made a fresh growth of 85·0 cm. One leaf at the 2nd and 3rd nodes of the scion and the same also on a similar shoot of the stock were inoculated, the control leaves being treated with water only. The leaf at the 2nd node was only about one-quarter expanded. By the 14th day the leaf at the 2nd node of the scion was fully and heavily infected, and bore very numerous, dense powdery patches, while the control leaf was free; the leaf at the 3rd node remained uninfected, as also the control leaf at that node. No infection resulted on the stock.

Exp. 3 *a*. Four leaves at the 2nd and 3rd nodes of the scion (Wild Hop A), one leaf at the 2nd node, and two leaves at the 3rd node of the stock (No. 310) were inoculated. In the case of both stock and scion, the leaves were at the same stage of development, viz. at the 2nd node only just expanding and at the 3rd node about one-quarter expanded. By the 6th day all the four leaves of the scion were fully infected and showed an abundant mycelial growth and young conidiophores densely clustered. No infection occurred on the stock.

Exp. 4 *a*. Four leaves at the 2nd and 3rd nodes of the scion (B 18) were inoculated and by the 12th day were fully infected. No inoculation of the stock was made.

Exp. 4 *b*. The above scion was used again 15 days later. Four leaves at the new 2nd and 3rd nodes were inoculated; the former were very small (1·5–2·0 cm. long), and the latter about half expanded. By the 10th day the leaves at the 2nd node were fully infected and those at the 3rd node weakly infected.

Exps. 5 *a and* 6 *a*. Two leaves at the 2nd and 3rd nodes respectively of the two scions (B 18) and one leaf at the 2nd and 3rd nodes respectively of the stocks (both unnamed immune) were inoculated. By the 7th day all the four leaves of the scions were fully infected. No infection of the stocks resulted.

Exp. 7 *a*. Four leaves at the 2nd and 3rd nodes of the scion (B 18) were inoculated. By the 19th day both the leaves at the 2nd node were so heavily infected with an almost continuous growth of dense powdery conidiophores that a cloud of conidia was dispersed on the plant being touched; the leaves at the 3rd node bore only small clusters of conidiophores spread over the surface of the leaf. No inoculation of the stock was made.

General Discussion.

On the subject of the study of the nature of varietal immunity in plants, we may quote here the remarks made by Roach (1) with reference to varieties of potatoes immune from wart disease: "There is singularly little knowledge however of the general nature of this immunity. It may for example be conferred by a definite substance not present in the susceptible varieties, or on the other hand it may be due to the absence in the immune varieties of a substance which makes growth of *Synchytrium endobioticum* possible. If either of these suppositions be correct, this substance may be produced throughout the whole plant or it may be produced in one part and translocated thence to the remaining tissues. The possibility that the difference between 'immunes' and 'susceptibles' is the result of slightly different chemical groupings in the protoplasm in the two cases, does not seem to have been explored."

As regards immunity from *Sphaerotheca Humuli*, the results of our experiments (Tables I and II) in which a considerable number of composite plants were built up by grafting, viz. 7 plants of susceptible varieties grafted on immune, and 13 plants of immune varieties grafted on susceptible, show that the original degree, either of resistance or susceptibility, in the ten varieties used remained unchanged in the scion. No evidence was obtained of decreased infection of the susceptible scion when grafted on immune stocks. Conversely, shoots of immune varieties grafted on susceptible stocks remained immune. For both these types of experiment a variety such as B 18, with its known extreme susceptibility to the mildew, was very suitable and make the results obtained the more noteworthy. It is to be remarked further that among the immune varieties used, two, viz. Golden Hop and No. 310,

have been observed to show in the greenhouse a temporary breakdown of immunity which permits of the production of a weak growth of conidiophores on the leaves. These cases have been described above (p. 263) and the cause of the loss of immunity has been tentatively ascribed to the effect of weather conditions (possibly low temperature).

In the case of another immune variety used, viz. No. V 93, although the plants grown in the greenhouse have always shown complete resistance, a slight susceptibility in plants grown in the open has occasionally been observed in the late autumn ((3), p. 151). Of these three varieties, scions of Nos. 310 and V 93 showed in the grafting experiments complete resistance to the mildew, while the scions of the Golden Hop remained immune in five of the experiments and in two became "sub-infected" in exactly the same manner as did certain ungrafted plants of that variety in the greenhouse[1]. Such plants as the above three varieties may be considered as especially suitable for testing the effects of grafting because an interference in their metabolism, such as may apparently be produced by low temperature, results in a temporary loss of complete resistance to the mildew. It is all the more interesting, therefore, to find that the process of grafting with the possibility of the scion experiencing an alteration in the supply of substances from a susceptible stock, affects in no way the varietal reaction to the attacks of the mildew.

Summary.

1. Twenty composite plants were built up by grafting either a scion of an immune variety of hop on a susceptible stock, or *vice versa*. Inoculation of young leaves of the scion, after a considerable growth of the latter had taken place since grafting, showed that no change had been effected in the varietal immunity or susceptibility in relation to *Sphaerotheca Humuli*.

2. In the above experiments, scions of six different immune varieties were grafted on stocks of four different susceptible varieties and scions of four different susceptible varieties were grafted on stocks of five different immune varieties.

3. The results obtained render it improbable that immunity or susceptibility to *S. Humuli* is connected with any translocatable substance in the hop plant.

[1] See above, p. 263.

Table I.

Grafting Experiments with Immune Scions on Susceptible Stocks.

Exp. No.	Susceptible stock	Immune scion	Date of graft	Date of records	Total height of scion (cm.) from ground	Pairs of leaves expanded above graft	Node above graft where leaf inoculated	Distance of node from growing point (cm.)	Result of inoculation on	
									Stock	Scion
1	Wild Hop C	Golden Hop	Mar. 27	Apr. 13	18·5	7				
(*a*)	,,	,,		,, 29	27·5		7th	8·5	+4	?+2
(*b*)	,,	,,		May 10	43·5		10th 11th	14·0 8·0	+2	−1 −1
2	Wild Hop C	Golden Hop	,, 27	Apr. 13	20·8	7				
(*a*)	,,	,,		,, 30	35·2		7th 8th	14·5 10·8	+4	?+2 ?+2
(*b*)	,,	,,		May 19	85·0		13th 14th	23·0 13·0	+2	−1 −1
3	Wild Hop C	Golden Hop	,, 27	Apr. 13	13·2	6				
(*a*)	,,	,,		May 5	39·0		7th 8th	21·0 17·6	+4	−2 −2
(*b*)	,,	,,		,, 25	80·0		15th 16th	15·0 7·0	−1 +1	−1 −1
4	Wild Hop C	Golden Hop	,, 27	Apr. 13	23·4	6				
(*a*)	,,	,,		May 5	31·0		7th	6·6	+2	−2
5	Wild Hop A	310	May 13	,, 13	13·8	3				
(*a*)	,,	,,		June 8	55·0		9th	15·0	+2	−2
6	Tutsham	310	,, 18	May 18	28·1	4				
(*a*)	,,	,,		,, 28	47·5		6th	12·5	+2	−2

7	B 18	V 93	Mar. 12	Mar. 29	15·6	4				
(*a*)	,,	,,		May 5	79·0		8th 9th	30·0 20·0	(0)	−2 −2
(*b*)	,,	,,		,, 21	115·0		12th 13th	24·0 14·0	(0)	−2 −2
8	B 18	H 24	,, 12	Mar. 29	10·1	3				
(*a*)	,,	,,		May 5	32·0		6th	11·5	+1	−2
(*b*)	,,	,,		,, 25	89·0		10th	18·0	+1	−1
9	B 18	H 24	,, 12	Mar. 29	10·4	3				
(*a*)	,,	,,		May 5	33·0		5th	13·6	+1	−2
(*b*)	,,	,,		,, 25	100·0		11th	12·0	(0)	−1
10	B 18	Unnamed immune	,, 13	Mar. 29	15·5	4				
(*a*)	,,	,,		May 6	33·0		6th 7th	6·8 3·4	+4	−2 −2
(*b*)	,,	,,		,, 21	46·0		9th	—	+2	−2
11	B 18	Unnamed immune	,, 13	Mar. 29	11·8	3				
(*a*)	,,	,,		May 6	41·8		5th 6th	22·5 12·5	+4	−2 −2
(*b*)	,,	,,		,, 17	75·0		8th 9th	27·0 14·0	+2	−1 −1
(*c*)	,,	,,		June 3	142·0		14th 15th	29·5 14·5	+3	−2 −1
12	B 18	Unnamed immune	,, 13	Mar. 29	25·4	4				
(*a*)	,,	,,		May 10	38·0		6th 7th	7·4 4·0	+4	−2 −2
(*b*)	,,	,,		,, 25	62·5		10th	10·5	+2	−2
13	B 18	H 30	,, 12	Mar. 29	12·0	2				
(*a*)	,,	,,		May 7	24·4		4th 5th	3·2 1·0	(0)	−2 −2

Table II.

Grafting Experiments with Susceptible Scions on Immune Stocks.

Exp. No.	Immune stock	Susceptible scion	Date of graft	Date of records	Total height of scion (cm.) from ground	Pairs of leaves expanded above graft	Node above graft where leaf inoculated	Distance of node from growing point (cm.)	Result of inoculation on	
									Stock	Scion
1	Golden Hop	Tutsham	Apr. 20	May 6	24·2	5				
(*a*)	,,	,,		,, 18	49·2		6th 7th	24·5 17·0	−2	−2 +2
2	310	Wild Hop B	,, 15	,, 6	24·8	7				
(*a*)	,,	,,		,, 18	65·0		8th	24·1	−2	+2
(*b*)	,,	,,		June 3	150·0		13th 14th	29·0 15·5	−2	−1 +1
3	310	Wild Hop A	May 13	May 13	23·5	5				
(*a*)	,,	,,		June 11	92·0		10th 11th	34·5 19·3	−3	+2 +2
4	V 93	B 18	Mar. 12	Mar. 29	11·2	3				
(*a*)	,,	,,		May 5	49·0		5th 6th	22·0 14·0	(0)	+2 +2
(*b*)	,,	,,		,, 21	59·0		8th 9th	12·0 7·0	(0)	+2 +2
5	Unnamed immune	B 18	,, 13	Mar. 29	6·5	2				
(*a*)	,,	,,		June 10	58·0		12th	18·5	−1	+2
6	Unnamed immune	B 18	,, 13	Mar. 29	16·8	3				
(*a*)	,,	,,		June 10	76·0		15th	9·0	−1	+2
7	II 30	B 18	,, 12	Mar. 29	12·5	3				
(*a*)	,,	,,		May 7	58·2		6th 7th	31·0 19·0	(0)	+2 +2

SALMON & WARE.—Grafting Experiments with Varieties of Hops (pp. 276—289).

BIBLIOGRAPHY.

(1) Roach, W. A. (1923). Studies in the Varietal Immunity of Potatoes to Wart Disease (*Synchytrium endobioticum* (Schilb.) Perc.). Part I. *Ann. App. Biol.* x, 142.

(2) Salmon, E. S. and Ware, W. M. (1925). Virus Diseases and the Grafting of the Hop. *Gardeners' Chronicle*, May 9th, lxxvii. 320.

(3) Salmon, E. S. (1921). On Forms of the Hop (*H. Lupulus*) resistant to Mildew (*S. Humuli* (DC.) Burr.); V. *Ann. App. Biol.* viii, 150, 151, 156.

EXPLANATION OF PLATE XVIII

The susceptible variety of Hop, B 18, with two shoots. Right, ungrafted; left, a shoot on which a scion of an immune variety was grafted at (*a*) on March 13th. After vigorous growth had taken place, two young leaves of the same age on each shoot were inoculated on May 21st. The immune scion has completely resisted infection (*b*), whereas the stock has become fully infected (*c*). Photographed June 1st. (See Table I, Exp. 10 *b*.) $\frac{1}{3}$ Natural size.

(*Received February 26th*, 1927.)

RHIZOCTONIA "FOOT-ROT" OF THE TOMATO[1]

By T. SMALL.

(*Experimental and Research Station, Cheshunt, Herts.*)

Epidemics of foot-rot of young tomato plants caused by *Rhizoctonia solani* (Kühn) have inflicted considerable losses upon some growers in Guernsey. The disease appears shortly after planting in the houses and results in the formation of brown lesions at soil level which gradually extend and finally cause the plant to fall over. In the present investigation the effect of various cultural devices and also of soil fungicides has been tested.

I. Inoculation Experiments.

These experiments were conducted in a glasshouse having an average temperature of 21° C. and tomato plants in four-inch pots were used. All factors were kept as constant as possible with the exception of that under consideration. Inoculations were made from cultures on Coons agar, the inoculum being so placed that the fungus was compelled to grow through half an inch of soil before reaching the plants. Cultures of the same age were used for all the plants in any one experiment; this precaution was necessary because a decided loss of virulence, indicated by meagre production of sclerotia, occurred in pure culture which was however regained by passage through a host plant. Controls received culture medium only and remained healthy in all cases. At the conclusion of each experiment, the duration of which varied from 7 to 14 days, the plants were examined and re-isolations were made from representative members. To estimate the extent of the disease the following degrees of infection were adopted: none; fair, lesion $\frac{1}{4}$ in. to $\frac{1}{2}$ in. long; bad, lesion $\frac{1}{2}$ in. or more in length. Preliminary experiments, made with the object of finding a suitable variety for later work, showed that all the varieties tested were equally susceptible and as Ailsa Craig was available it was used for all subsequent experiments.

[1] Portion of a thesis approved for the Degree of Master of Science in the University of London.

1. *The relation of soil moisture and soil type to the disease.*

A knowledge of this relation is especially desirable because the amount of water supplied and the method of application can be varied at will under nursery conditions. Inoculated plants were submitted to different moisture conditions, the details of which with the results obtained are shown in Table I.

Table I.

Degree of infection	Daily treatment per four-inch pot			Soil type	
	No water	50 c.c.	100 c.c.	Sandy	Clay
None	21	2	2	6	3
Fair	8	11	3	5	—
Bad	1	17	19	6	14
Total No. of plants	30	30	24	17	17

These results suggested that planting in furrows as practised by some growers would render the plants more liable to infection and this was confirmed experimentally.

The above evidence indicated that the disease is least prevalent on dry, open soils and emphasised the danger of furrow planting.

2. *The effect of temperature upon the disease.*

To determine the optimum temperature for infection inoculated plants were placed in different positions, the maximum, minimum, morning and afternoon temperatures of which were recorded each day. The temperature variation in these positions was no greater than is usual in large glasshouses and does not therefore invalidate the practical value of the results. Consideration of the data given in Table II shows that severe infection occurred between 16·1° C. and 20·7° C. and later experiments confirmed that below and above these temperatures respectively, the degree of infection diminished.

Table II.

Temperature in ° C.			No. of plants	Degree of infection		
Absolute minimum	Absolute maximum	Average		None	Fair	Bad
19	42	27·0	21	13	6	2
11	34	20·7	21	6	7	8
9	24	16·1	21	1	2	18
2	12	7·4	15	13	2	—

Recent work at Cheshunt has shown that harmful results occur when tomato plants are set out at a soil temperature less than 13·8° C. (57° F.) with the result that most growers do not plant until the soil is sufficiently warm. Further, in commercial practice soil watering is withheld until the fruit on the second truss is swelling, a period of about 60 days after planting, because earlier watering is prejudicial to the setting of the earlier flowers. It follows that the soil must be reasonably moist when planting. Thus the temperature and moisture conditions of the soil at planting time are normally favourable to infection by *Rhizoctonia*. Growers have some difficulty in obtaining a soil temperature higher than 15·5° C. (60° F.) at planting time and indeed this temperature is attained only by heating the empty houses for 14 to 20 days previously. Therefore any control measure which depends upon soil temperatures higher than 15·5° C. is almost impracticable. It is also difficult to lower the moisture content of the soil at planting time but growers should remember that dry open soils are least favourable to *Rhizoctonia*.

3. *The effect of manurial treatment upon the disease.*

An attempt was made to see how infection by *Rhizoctonia* was affected by the various manures used in tomato culture.

The abundant fungal growth produced upon sterile stable manure suggested that this material might be expected to increase the amount

Table III.

Manure	Percentage in soil	No. of plants	Degree of infection		
			None	Fair	Bad
Stable manure	None	17	2	7	7
,,	3·0	17	1	3	13
,,	6·0	17	2	—	15
Ammonium sulphate	None	38	2	16	20
,,	0·5	38	14	16	8
Complete artificials*	None	10	—	1	9
,,	1·5	10	5	4	1
Complete artificials minus potash	1·0	10	2	5	3
Complete artificials minus nitrogen	1·0	10	—	1	9
Complete artificials minus phosphate	1·0	10	3	6	1

* Complete artificials includes potassium sulphate, superphosphate and ammonium sulphate.

of infection and it is clear from the results of confirmatory experiments given in Table III that this is actually the case.

The results of the fertiliser experiments were not conclusive but suggested that a top dressing of sulphate of ammonia may reduce the amount of disease, and field experiments are being arranged to test this assumption on a larger scale.

Lime used in various quantities from 1 to 10 lb. per square yard scarcely affected the degree of infection.

II. Soil Sterilisation.

1. *Summer treatment.*

An attempt was made to discover a fungicide which would enable the grower to re-plant the infected areas at once. For this purpose solutions of the following compounds were tested using 100 c.c. of a strength which just failed to kill the plants: Cheshunt compound, copper sulphate, sulphuric acid, formaldehyde uspulun, ammonium polysulphide, ammonium hydroxide, ammonium carbonate, ammonium sulphate, ammonium chloride and ammonium nitrate. Only uspulun appeared to have any effect upon the disease and in a final test with this fungicide, using a 0·25 per cent. solution in sufficient quantity to saturate the soil, 13 out of 16 plants escaped infection whilst all the controls were severely diseased.

2. *Winter treatment.*

It is obvious from observation in commercial nurseries and experiments conducted during the course of this investigation that the organism causing this disease overwinters in the soil and therefore on infected nurseries it is necessary to sterilise the soil at the end of the season. Laboratory experiments sufficed to show that infected soil, even when large sclerotia were present, can be freed from *Rhizoctonia* by steaming for one hour at 96° C. and that when such soil is re-infected after sterilisation the disease spreads much more rapidly than in non-steamed soil. Baking in a standard oven gave similar results. Steam sterilisation of infected soils on nurseries has repeatedly given freedom from this disease.

Some growers cannot be persuaded to employ steam sterilisation because they live in localities where boilers cannot be hired and they do not wish to incur initial cost of the necessary apparatus. For this reason a number of likely chemical compounds was tested to see if the disease could be controlled by the use of chemical sterilising agents. For this purpose copiously infected soil was passed through a $\frac{1}{4}$ in. sieve

prior to treatment with fungicides which were diluted at the rate of 100 c.c. per pound of soil to be treated and were applied as evenly as possible. Soil treated with volatile compounds was covered for two days. Controls received water instead of fungicide and when planted infection always occurred. The following compounds proved to be valueless: cresylic acid (0·36 per cent.), lysol (0·08 per cent.), potassium dichlorphenol (0·056 per cent.), copper sulphate (1·32 per cent.), flowers of sulphur (0·5 per cent.) and potassium permanganate (0·27 per cent.).

Promising compounds were tested again and the results, given in Table IV, showed that in no case was the soil freed from the disease although the treatment was such that it would be too costly for use on a commercial scale. When it is remembered that these experiments were carried out carefully on a small scale it seems reasonable to conclude that control by these fungicides is less reliable than steaming and is indeed rarely possible under nursery conditions.

Table IV.

Sterilisation of infected soil by fungicides.

Compound	Percentage in soil	Application per acre in tons	No. of plants	Number infected
Formaldehyde	0·250	2·50	10	3
Phenol	0·220	2·20	10	2
Potassium dichlorcresylate*	0·032	0·32	10	2
Potassium dichlorphenate*	0·065	0·65	15	3
Ammonium carbonate	0·880	8·80	15	4
Ammonium hydroxide	1·100	11·00	10	4

* Viable sclerotia were found on the sides of some pots containing healthy plants.

III. Control Methods.

As a result of this investigation the following methods of control may be suggested:

1. Diseased plants and the soil immediately surrounding them should be removed, care being taken to ensure that no soil is transferred from the diseased area to other parts of the house, and the hole thus made should be treated with a 0·25 per cent. solution of uspulun. The area may then be re-planted and the plants given a top dressing of ammonium sulphate.

2. Dry open soil conditions should be maintained by periodic hoeing, and, if possible, by reducing the water supply and raising the temperature.

3. The soil should be steamed after uprooting and removing the plants at the end of the season.

4. Weeds in and about the glasshouses must be destroyed, as experiments have indicated that these may serve as hosts and thus help to maintain the virulence of the fungus.

The author wishes to acknowledge his indebtedness to Dr W. F. Bewley, Director of the Experimental and Research Station, Cheshunt, for advice and helpful criticism during the investigation and the preparation of this paper.

IV. Summary.

1. A "foot-rot" disease of young tomato plants from Guernsey caused by *Rhizoctonia solani* has been investigated.

2. The disease is least prevalent on dry open soils; it is most severe at temperatures ranging from 16° C.–20° C.

3. The degree of infection was increased by stable manure; slightly decreased by ammonium sulphate; apparently unaffected by lime, potassic and phosphatic manures.

4. Soil sterilisation by heat was the most effective and reliable means of controlling the disease. During the season the disease was checked by uspulun.

5. Control methods are suggested.

(*Received April 4th*, 1927.)

MANGEL SCAB—ITS CAUSE AND HISTOGENY

By W. A. MILLARD, B.Sc.
(*Lecturer in Agricultural Botany and Adviser in Mycology, University of Leeds*),
and FRED BEELEY, B.Sc.
(*Field Officer and Mycologist to the Rubber Research Institute, Federated Malay States*).

(With Plates XIX–XXII and 1 Text-figure.)

Introduction.

The causative organism of mangel scab has long been recorded as identical with that of common scab of potatoes and the work of previous investigators leaves no room for doubt that the two diseases are closely allied. It is moreover a common experience to find that where potatoes are scabbed, mangels and beet and also turnips grown on the same soil are similarly affected. Since, however, Wollenweber (6) and Millard and Burr (4) have shown that potato scab may be produced by a variety of *Actinomyces* species, the assumption of identity between the two diseases becomes meaningless unless, indeed, it should transpire that each of the *Actinomyces* species producing scab on potatoes is able also to produce it on beet. It has also become obvious that, at the time of the early investigations of beet scab, no accurate comparison could be made between *Actinomyces* strains isolated from the two diseases. In this connection, however, it is interesting to note that in 1904, Krüger (2), working on what he called "girdle" scab of sugar beet, stated that the *Actinomyces* strains which he isolated were not identical with the Thaxter organism of potato scab, and again, in 1915, Lutman and Johnson (3) recorded the isolation of eight strains of *Actinomyces* drawn from beet scab, five of which were found to be pathogenic. One of these was decidedly more virulent than the remaining four. In spite of these results, the identity of the organisms of potato and beet scab was tacitly accepted.

The morphological differences between the disease in its most noticeable forms on the two hosts are obvious even to the casual observer. In the potato the scabs are frequently sunken (pitted) or, if superficial,

consist of a mass of cork, whereas, in the beet the scab frequently forms large knob-like protuberances up to 20 mm. across and rising 6 mm. or more above the surface. It is plain that this morphological difference may be due either to different organisms or to innate differences between the two host plants in their reaction to the invading parasite, or again, to a combination of both factors. The present work was undertaken with the object of finding the causative organism or organisms of beet scab and of ascertaining more precisely the manner in which the scabs developed under their invasion.

Present Work.

Mangels of the Golden Tankard and Red Intermediate varieties were obtained from a field at Strensall where the soil is a very sandy loam and yields badly scabbed crops of potatoes. The mangel crop was severely scabbed and photographs of typical roots are shown in Plate XIX, figs. 1 and 2. It will be observed that the scabs are in general confined to that portion of the root which is at or below soil level. Examination of these specimens revealed the fact that, in addition to the large bulging scabs, another type of scab was also present. This consisted of an irregular cavity filled with brown rotting tissue covered when young, by a plate of hard black suberised tissue. Other scabs in the neighbourhood of these shallow pits consisted of an irregular, corky mass rising slightly above the surface of the root in an uneven manner very different from the more noticeable bulging scab and may possibly have been older stages of the pitted variety. They are best seen in the specimen shown in Plate XIX, fig. 2.

Isolation of the Causative Organisms.

(I) *Pitted Scab.*

The mangels were first thoroughly washed and allowed to dry. A typical scab was then selected and wiped with a camel hair brush dipped into methylated spirits. With the aid of a sterile scalpel the upper part of the scab was removed whilst the tissue below was mulched into a pulp. A small portion of this pulp served as inoculum and with it plates of nutrient potato agar were poured. The latter were incubated at 24° C. for 7 days and the colonies which had then grown in each dilution plate were found to be almost entirely *Actinomyces*. All the colonies appearing in the plates of the higher dilutions stained the

medium a dark brown and none produced aerial mycelium. As no differences could be observed between them pure cultures were made from only two; these eventually proved to be duplicates and form Strain 6 of this paper.

(II) *Raised Scab.*

From subsequent work on the structure of raised scab it seems very possible that there are two variants of this type and these will be later described. At the time of making these isolations no such distinction had been drawn, but the inoculation material from which the cultures were obtained was taken from a bulging scab with gently sloping sides which afterwards came to be known as "mound" scab. The same procedure was followed as in the case of pitted scab, but in the first two attempts at isolation the plates showed large numbers of bacteria and very few *Actinomyces*. Finally, however, the inoculum was taken from the periphery of the scab and here the results obtained were much more satisfactory. Bacterial and Actinomycetal colonies were present in approximately equal numbers. None of the *Actinomyces* colonies stained the medium, but other variations were noticed between them and eight were isolated into pure culture. A preliminary examination of the growth of these was made on calcium malate glycerine agar, glycerine synthetic agar, dextrose synthetic agar, and nutrient potato agar, and by this means three of the eight strains were judged to be duplicates; the cultures were thus reduced in number to five (Strains 1, 2, 3, 4 and 5), all of which proved to be different from one another.

Inoculation Experiments.

The task before us consisted first in testing Strain 6 (from pitted scab) and the five strains (Nos. 1–5) from knob scab *individually*, and for this purpose, it was thought desirable to use duplicate pots each containing three plants for inoculation with each strain. Secondly, it was necessary to carry out inoculations with as many *combinations* of the five strains (Nos. 1–5) as possible. It was obviously impossible to try all possible combinations since, if for each inoculation a maximum of only three roots were used, the number of plants required would have been too large to cope with. The multiple inoculations were therefore limited to the possible combinations taking two strains at a time. From previous experience with potatoes it seemed that the most satisfactory method of inoculation in such an experiment was that of soil inoculation, but, again in order to spare the labour involved, it was determined to

use this method only for the *individual* inoculations and to carry out the *dual* inoculations by direct application of the inoculum to the growing roots. It was thought that in addition to the inoculations so arranged, it would be of great interest to test the effect of a potato scab producing *Actinomyces* species on mangels and for this purpose the very virulent species *A. scabies* (Thaxter) Güssow, emend. M. and B., was selected and included in the series of direct inoculations. Finally, since before the direct inoculations were carried out it became obvious that Strain No. 6 was proving positive in the soil inoculations, a direct inoculation with this strain was also made.

For the soil inoculations 14 large plant pots were filled with soil which had previously been mixed with sand in the proportion of 2 parts loamy soil to 1 part sand. The mixture thus obtained was a light sandy loam in which *Actinomyces* are known to thrive. Its lime requirement was nil. The pots were sterilised at $3\frac{1}{2}$ atmospheres' pressure for 15 minutes and were then transferred to a previously sterilised greenhouse where the temperature varied from 18° to 20° C. by day and from 14° to 16° C. by night. Inoculations of the soil were made with potato plug cultures of the various strains, using 20 cultures to each pot. These were buried to a depth of 4 in. and then cut and mixed with the soil by means of sterile scalpels.

The mangel plants required for the direct inoculations were grown in large boxes 6 ft. long by 1 ft. deep filled with unsterilised soil.

The varieties of mangel selected for both series of inoculations were Yellow Globe and Red Intermediate. The seed was first sterilised in a $\frac{1}{6}$th per cent. solution of formaldehyde for 2 hours after which it was washed in distilled water and dried under cover on a porous plate. In order to ascertain whether this treatment had affected the germination of the seed adversely, germination tests were carried out before sowing. Yellow Globe Mangel gave a germination capacity of 70 per cent. and Red Intermediate 55 per cent. In sowing the seed in the pots, therefore, five seeds were placed in each of three shallow holes in the soil of each pot, whilst, in the boxes similar sowings were made at distances of 8 in. apart. Each pot was given 15 gm. of a sterilised mixture of artificial manure consisting of 1 part sodium nitrate, 3 parts superphosphate and 3 parts kainit and the boxes were given proportionate quantities of the same mixture. When the seedlings were in their 3rd or 4th leaf they were singled, so that, finally, each pot contained three roots, one red and two yellow, and in the boxes similar groups of three were made by inserting thin strips of wood to a depth of 6 in. in the soil.

Direct inoculation of the plants in the boxes was carried out when the diameter of the bulbed roots was from 3 to 4 in. Each group constituted a unit for one mixed inoculation. The inoculum used was prepared by mixing emulsions made with sterile water of the two strains of *Actinomyces*. A band of damp cotton wool was tightly secured around each bulb at soil level and the emulsion was then poured on the root so that it lodged between the surface of the root and the cotton wool. The roots were not injured in any way before or after adding the inoculum. During the further period of the experiment the cotton wool around the roots was moistened from time to time with sterile water. It will be seen that in this procedure the possibility of natural infection of the roots from the soil was not excluded. One group of three roots was, however, left as control and here sterile water in place of an inoculum was poured between the cotton wool and the root. At the time of making the direct inoculations the roots were perfectly free from scab and no scab developed on the three controls during the experiment. Moreover, the great majority of these dual inoculations gave negative results and we feel justified, therefore, in accepting such as were positive. These were abundantly confirmed by the soil inoculation results.

It may be of interest to record that every plant left in the pots and

Results of Inoculation Experiments.

Series 1. *Soil Inoculation.*

No. of pot	Inoculated with *Actinomyces* strain No.	Result
1	1	Negative
2	1	,,
3	2	,,
4	2	,,
5	3	,,
6	3	,,
7	4	Positive. Producing mound scabs from 5 to 15 mm. diam. on Yellow Mangels only
8	4	Positive. Ditto
9	5	Negative
10	5	,,
11	6	Positive. Producing small dark pitted scabs on both Red and Yellow Mangels and also forming numerous scab nodules on the true roots. Some superficial scab was shown on the upper part of one root
12	6	Positive. Ditto
13	Control	Negative
14	,,	,,

Series 2. Direct Inoculation.

Group of roots No.	Inoculated with *Actinomyces* strain Nos.	Result
1	1+2	Negative
2	1+3	,,
3	1+4	Positive. Very small raised scabs
4	1+5	Negative
5	2+3	,,
6	2+4	Positive. A few minute scabs of indefinite type
7	2+5	Negative
8	3+4	,,
9	3+5	,,
10	4+5	,,
11	6	Positive. One large pitted scab on the Yellow Mangel with numerous nodules on the upper rootlets
12	*A. scabies*	Positive. Small brown pitted scabs on all three roots

in the boxes after singling formed a good bulbous root which in some cases measured as much as 4 in. in diameter—this, too, during the period from December to July.

The results from both series of inoculation experiments are given in the above table.

From these results it is clear that of the five strains isolated from mound scab, one only, Strain 4, was pathogenic. Where used under the conditions of soil inoculation this strain produced scabs which were unmistakably identical with the mound scab from which it was derived. A photograph of one of the infected roots from Pot 7 is given in Plate XX, fig. 3. In the mixed inoculations the same strain in combination with Strain 1 and with Strain 2 also gave weak positive results but proved negative in combination with Strains 3 and 5. It is possible that at the time of these direct inoculations the roots were too old for infection to take place or that where it did occur the disease could not make headway. Strain 6, derived from the pitted scab, proved to be pathogenic in soil inoculations and also to a somewhat lesser extent in the direct inoculations. In the latter, only the Yellow Mangel was attacked, but in the former both Red and Yellow varieties showed a number of small dark pitted scabs closely resembling the original. Some of the older scabs consisted of cork tissue raised unevenly above the surface, similar to those which, as already mentioned, occur on the naturally infected mangel. Again, on one root there was a considerable amount of superficial scab on the upper part. This modification is easily accounted for

when the development of this type of scab has been studied (see later section on the histogeny of pitted scab) since here, owing perhaps to the relatively dry conditions, the parasite has been unable to penetrate the first layer of cork laid down and the tissue above has dried off.

A further and more important result arising from the soil inoculations made with Strain 6 was that the true terminal and lateral roots were also severely attacked by the organism. This attack showed itself in the form of numerous irregular, dark brown nodules of scab tissue scattered over the surface. The fine rootlets were similarly attacked for the most part at their bases where the nodules coalesced to form a slightly raised mass extending along the two longitudinal rows. This infection of the roots of mangel plants has not so far as we are aware been previously recorded and is of special interest in view of a similar infection which has been shown by Millard and Burr(4) to occur in the potato plant. A photograph of one of the affected roots from Pot II, Series 1, is shown in Plate XX, fig. 4. Lastly, from Series 2, Group 12, the interesting result was obtained that the potato scabbing organism *A. scabies* (Thaxter) Güssow, emend. M. and B., was also pathogenic and produced scabs identical in appearance with those of Strain 6. The *Actinomyces* Strains 4 and 6 were easily re-isolated from the artificially produced raised and pitted scabs respectively and are therefore regarded as the causative organisms of these two types.

Definition of the Actinomyces *Strains* 4 *and* 6.

The following media were selected for culturing these two organisms, the *p*H being indicated in brackets: saccharose synthetic agar (6·8), glycerine synthetic agar (6·8), dextrose synthetic agar (6·8), calcium malate glycerine agar (7·0), dextrose (asparagin) agar (Krainsky) (6·8), nutrient potato agar (7·0), gelatin (to 7·4), egg albumen agar (7·0), potato plug, mangel plug, tyrosin agar (6·9), starch nitrate agar, saccharose synthetic solution (6·8), glycerine synthetic solution (6·8), glucose broth (7·1), brom-cresol milk (7·0). With the exception of gelatin (grown at room temperature) the cultures were incubated at a temperature of 24° C. for 4 weeks. In addition to the above cultures nitrate reduction and starch hydrolysis tests were carried out and also tests for growth under anerobic conditions and at a temperature of 37° C. The methods adopted in sub-culture and in recording cultural changes were those recommended by Millard and Burr(4) and comparison with the species defined by them was thus easily possible. Strain 6 proved to be identical with *A. scabies* (Thaxter) Güssow, emend. M. and B., thus

proving an identity between potato and mangel scab in respect of the pitted type of this disease. It is interesting to recall that the organism in question is that which produces root nodules on potatoes as already mentioned. The external effects produced by it on its respective hosts are therefore almost identical. No parallel could be found between Strain 4 and any recorded species and since also the cultural characteristics of this strain are very definite it has been given specific rank with the name of *Actinomyces tumuli*. A description of the more characteristic cultures of this species are given at the end of this paper, and a photograph of a few of these is shown in Plate XXI, fig. 5.

The Structure of Raised and Pitted Mangel Scabs.

An account is first given of the external features of these two types of scab.

(1) *Raised Scab.*

This consists of a mass of soft tissue 5–20 mm. in diameter rising 2–6 mm. from the surface of the mangel. The sides of the scab are fairly smooth but the upper surface may be roughened with small black patches of skin-like tissue which easily flake off. In some of the scabs the sides rise at right angles to the mangel surface and may even be waisted in the manner of a door handle; the surface is then flat or concave. In others, the scab forms a mound with gently sloping sides and the surface is convex. It seems possible then that raised scab should be subdivided into two types which we may call knob and mound scab, and detailed work on their mode of development and internal structure lends support to this view. If it be correct, then an interesting analogy is provided between the two types of raised scab arising on the mangel and those recorded by Millard and Burr (4) on the potato and ascribed by them to infection by different species of *Actinomyces*. In both mound and knob scabs a depression in the centre of the scab marks the point of infection.

(2) *Pitted Scab.*

This closely resembles potato scab of the same type and needs no further description than that given in the earlier part of this paper.

Histogeny of Mangel Scab.

The minute anatomy of mangel scab is so dependent on the peculiar mode of growth of the mangel root that it seems advisable first to give an account of this as described by Eames and MacDaniels (1).

"The first cambium of the seedling forms a ring of bundles close about the primary xylem. A secondary cambium soon arises in the pericycle and this is followed in rapid succession by others arising similarly. All layers continue to function perhaps indefinitely, though more slowly after an early period of activity. The cambium apparently arises as a continuous band, but soon forms more or less separate bundles, bands of conjunctive parenchyma developing between the vascular strips. The position of each new cambium as it arises in the pericycle is such that it encloses a few layers of pericyclic cells. These rapidly multiply and build up a parenchymatous layer. Alternate bands of proliferated pericycle and of vascular bundles are thus formed. The former constitute the dark coloured and the latter the light coloured rings in the beetroot. The bundles are themselves largely parenchymatous, but a few lignified cells occur in the xylem. Growth continues through all layers, in the bundles apparently both by cambium activity and by proliferation of the parenchyma of the xylem and phloem."

Again, discussing the function of the pericycle in roots on page 231,

"The pericycle is commonly a persistent structure in roots even where secondary thickening is well developed. In such cases new cells are added by the division of the existing parenchyma so that the layer is not ruptured by the growth beneath it." "Its component cells are largely parenchymatous in their nature, and although they become permanent tissue early in the development of the root, they retain their ability to initiate new structures by the formation of secondary meristems." "It is in the pericycle that...in the majority of cases the first periderm layer is formed."

In regard to the formation of scab it will be seen that it is the capacity for proliferation of the parenchymatous cells of the pericycle and the possibility for a periderm layer to arise in this tissue which are of importance. It may be added that in a healthy mangel root the rind is composed of a cork layer from one to two cells thick.

Mound Scab.

Hand and microtome sections were first made of young scabs about 5 mm. in diameter. The sections were stained either with safranin and Delafield's haematoxylin or with Sudan III, and a microphotograph of one of these is shown in Plate XXII, fig. 6. The scab tissue consists almost entirely of a thick layer of degenerate and heavily suberised cortical tissue in which large cavities occur. Below this the outermost layers of the pericycle show obvious signs of the beginning of meristematic proliferation by the appearance of cross walls in the cells. No cork or cork cambium is as yet present. It is here indeed that the mangel differs so markedly from the potato. Priestley and Bebbington (5) find that whereas, in a cut potato a cork meristem arises under favourable conditions in a few days, the mangel and beet take from 3 to 5 weeks. A similar difference is found between these plants in the formation of cork in response to *Actinomyces* attack.

Older scabs of 10 mm. diameter were next sectioned and a photomicrograph of one of these is shown in Plate XXII, fig. 7. Here considerable proliferation of the pericyclic zone has taken place, which is greatest immediately below the seat of infection and decreases in proportion to the distance from this point. Consequently, the scab tissues take on an appearance of a mound with gently sloping sides. A cork layer has now been formed in the outermost layer of the pericycle, and as might be expected this is thickest at the central point of the scab where it shows 5–6 cells, and thinnest on the slopes where it shows only 1–2 cells. Above this cork there is, as before, a thick layer of degenerate and heavily suberised tissue which easily flakes off thus exposing a smooth cork layer. Lutman and Johnson(3) suggest that this easily removable dead tissue consists of a thickened layer of cork but this is entirely in disagreement with our experience and is indeed very improbable on physiological grounds. The small black plates often seen on the surface of these scabs consist of fragments of this debris still adhering to the cork. It may be noted that the bundles in the outer vascular ring below the scab are poorly developed.

Knob Scab.

Here it seems that cork formation takes place at a somewhat earlier stage in the development of the scab and is moreover greater. Plate XXII, fig. 8, shows a section through the centre of a knob scab where a plate of cork 6–8 cells thick has been formed immediately below the seat of infection. As in mound scab, increase in thickness of the pericycle ceases beneath the cork layer as soon as this has been formed, since further pressure from below would naturally tend to rupture it. Apparently, however, in knob scab even when cork has been formed, the irritation stimulus of the parasite is not exhausted and the pericyclic zone continues to proliferate with the result that there is a rapid upward and outward growth of the tissues on either side of the central cork plate. Thus the upper surface of the scab tends to become concave and the bulge of tissue to develop a waist. The pressure of this growth is often sufficiently great to rupture the normal surface cork layer. Eventually, a cork layer 1–2 cells thick arises in the outermost layers of this new pericyclic tissue along the steep slopes of the scab. If, as seems probable, the mound and knob scabs are caused by two distinct strains of *Actinomyces*, then there is little doubt that that causing the latter exerts the stronger irritation stimulus. A common feature of the invading organism in both scabs is that it is unable to penetrate deeply into the tissues

but having established itself spreads laterally through the cortical tissues.

A further interesting feature of knob scab is that one or more new vascular rings form in the proliferated pericycle of the scab tissue as seen in Plate XXII, figs. 8 and 9. The vascular elements in these adventitious rings are often, however, poorly developed. Lutman and Johnson (3) state that in raised scabs on garden beet "the protruding portion is due to an excess in growth of the rings directly under the infected spot," but in the field mangels with which we have worked we have not found this to be the case. Indeed, it seems clear from the Fig. No. 2 given by these workers that the protruding portion of the scab shown has originated by proliferation of the pericycle. It must be remembered, that at the time this work was published the view of De Bary was generally accepted that each zone of tissue, whether vascular or parenchymatous, in the growing root originated entirely from the cambium of a vascular ring and this probably influenced the authors in the interpretation of their results. Again, whilst in the raised scabs we have examined, the scab tissues are definitely pericyclic in origin, a certain slight bowing out of the normal vascular rings in the direction of the scab does occur and it is not impossible that the new rings laid down in this scab tissue may link up with the former giving a fallacious appearance of common origin.

Pitted Scab.

This form of scab is radically different in its development from the raised types and approximates much more closely to the pitted scabs of potatoes. A section is shown in Text-fig. 1. The invading organism ramifies in the cortical parenchyma and then penetrates deeply into the tissues easily passing through the outer vascular ring. It reduces these tissues to a vacuolated pulpy mass. In the meantime, no proliferation of the pericycle takes place as in mound or knob scab but, on the other hand, a very active cork meristem arises in the pericycle from which a layer of cork up to 10 cells thick is ultimately formed. In the illustration given, this cork layer cuts across the outer vascular ring and it is thus clear that it is not formed from the normal cambium as suggested by Lutman and Johnson (3). Even this thick layer of cork does not suffice to check the organism which probably passes through it before suberisation can take place and frequently penetrates to the second ring of vascular bundles or even further.

It is thus obvious that the tissue destruction caused by pitted scab

is much greater than that of the more noticeable raised scabs. In older scabs of this type the scabs become slightly elevated above the surface by the upward pressure of successive cork layers arising below the first.

Text-fig. 1. Radial section through a pitted scab—drawn under the camera lucida. Magnification ×95. (*a*) Disorganised and vacuolated tissues of the cortex and outer pericycle. (*b*) Cork layer (the outer cells of which are strongly suberised) passing from the outer pericycle at the edges of the scab through the first vascular ring into the second pericyclic ring at the centre of the scab. (*c*) Penetration of the organism through the cork layer. (*d*) One bundle of the outer vascular ring. (*e*) Second vascular ring—so far, intact.

Summary.

It is shown that scab of mangels is of two distinct types—raised and pitted. The raised type may be further sub-divided into two which are called mound and knob scab respectively. These latter types appear to develop more commonly on yellow-skinned varieties.

Investigation of the development and structure of these scabs reveals a striking difference in their origin. In mound and knob scab the first response of the host to parasitic attack is an active meristematic proliferation of the outer pericycle which forms a mound-like protuberance. Some time afterwards—not less than a month—a cork meristem arises in the outer layers of this new pericyclic tissue and the mound becomes

capped with a layer of cork several cells thick at the apex and very thin over the slopes. In mound scab no further growth seems to occur but in knob scab the pericycle continues to proliferate and the new tissue, prevented from growing directly upwards, gives rise to the overhanging bulges so characteristic of this type. For the same reason the surface of this type of scab tends to become concave.

It is thus shown that raised mangel scabs are not formed from the cambium of the vascular rings. On the other hand, the normal vascular rings may bow out slightly towards the scab tissues and one or more adventitious rings of vascular bundles frequently arise in the proliferated pericycle of the scab tissue.

In both types of raised scab the outer dead tissues easily flake off thus exposing a smooth corky surface, but portions often remain adhering to this in small black patches. The point of infection in both scabs is marked by a central depression in the wart.

In pitted scab no proliferation of the pericycle takes place and thus, at first, a shallow pit is formed. A cork meristem then arises in the outer pericycle giving rise to a saucer-shaped cork layer up to 10 cells thick. This is frequently penetrated by the organism which may reach and destroy the first and possibly the second vascular rings at the seat of infection. Further and more deeply seated layers of cork then arise and the scab tissues are thereby forced upwards forming a wrinkled, corky scab slightly raised above the surface. Pitted scab formation in the mangel is thus very similar to that of the ordinary type of common scab in potatoes.

From mound scab a strain of *Actinomyces* has been isolated which reproduced the same type in inoculation experiments. The more outstanding cultural characteristics of this strain have been described and since it is new to the literature and appears to merit specific rank it has been named *Actinomyces tumuli*.

From pitted scab a strain of *Actinomyces* was isolated which also reproduced its own type in inoculation experiments. In addition, in these experiments the organism attacked the true roots and fibrous rootlets of the inoculated mangel plants on which it produced numerous characteristic, dark brown, nodular outgrowths.

This organism proved to be identical with *A. scabies* (Thaxter) Güssow, emend. M. and B. The same type of scab was produced in inoculation experiments with a stock culture of this organism.

Description of the more characteristic cultures of Actinomyces *Strain* 4—A. tumuli.

The cultures were examined at weekly intervals and the descriptions given below record the more outstanding changes which occurred during four weeks. The numbers used to indicate these changes often approximate to, but do not accurately stand for weekly changes.

Colours given in inverted commas are taken from Ridgway's *Color Standards and Color Nomenclature.*

Solid Media.

Saccharose synthetic agar.

Colonies. Small, with bowl-like projections into medium.
Growth. Fair, "Pale Smoke Grey" becoming darkly opaque.
Aerial mycelium. Arises on centre of colony; later, general. (1) White; (2) "Pallid Neutral Grey."
Guttation. Colourless drops leaving small black craters.
Pigment. None.

Glycerine synthetic agar. Plate XXI, fig. 5 (1).

Colonies. Raised centres with wide flat rims; round, forming hemispherical growth in medium.
Growth. Fair; later, good.
Aerial mycelium. Scant at first, borne centrally; later, general; white.
Guttation. At first numerous small colourless drops; later, coalescing to form sector-shaped drops; leaving a circle of black craters giving the colony a wheel-like appearance.
Pigment. (1) "Ecru Olive"; (2) "Tawny Olive."

Dextrose synthetic agar. Plate XXI, fig. 5 (2).

Colonies. Large round, with hemispherical growth in medium. The centres of the colonies become wrinkled and a flat margin develops.
Growth. Good, "Pale Smoke Grey."
Aerial mycelium. Arises in concentric rings round a dark bare centre; later, general, on margin only. White.
Guttation. None.
Pigment. (1) "Buffy Olive"; (2) "Tawny Olive."

Calcium malate glycerine agar. Plate XXI, fig. 5 (3).

Colonies. Finely fringed; hemispherical growth in medium.
Growth. Fair; later good. "Pale Smoke Grey."
Aerial mycelium. Annular at first; white; later, general, "Pale Smoke Grey."
Guttation. A ring of small droplets on each colony, leaving ring of craters.
Pigment. Faint brownish.

Dextrose asparagin agar (Krainsky). Plate XXI, fig. 5 (4).

Colonies. Raised, with bowl-like growth in medium, margins finely fringed.
Growth. Fair, later, good. "Pale Smoke Grey."
Aerial mycelium. (1) On fringe only, white; (2) general, "Pallid Neutral Grey."
Guttation. Arising after second week; minute colourless drops leaving minute craters.
Pigment. Slight. "Ecru Olive."

Nutrient potato agar. Plate XXI, fig. 5 (5).

Colonies. Umbonate with broad flat margin and bowl-like growth in medium.
Growth. Good, lustrous, slimy. "Pale Smoke Grey."
Aerial mycelium. None.
Guttation. None.
Pigment. None.

Gelatine.

Growth. Beaded with clump at base of liquefied basin.
Aerial mycelium. None.
Liquefaction. Stratiform, complete in two weeks, followed by digestion and production of clear liquid.
Pigment. None.

Egg albumen agar.

Growth. Good, ramifying into medium.
Aerial mycelium. Arises in centres of colonies, white; later, general, dull white to "Pale Smoke Grey."
Guttation. Small colourless drops leaving dark craters.
Pigment. None.

Potato plug. Plate XXI, fig. 5 (6).

Growth. Heavy, slimy, puckered, black. Later, a flat margin of "Greyish Olive" growth appears.
Aerial mycelium. None.
Colour of plug. Greyish brown.

Mangel plug.

Growth. Good, pale, translucent, raised.
Aerial mycelium. At first, scanty, white; later, abundant, "Pallid Mouse Grey."

Tyrosin reaction. Negative.

Growth under anaerobic conditions. None.

Starch plate. Positive. Width of hydrolytic zone complete 10 mm., incomplete 15 mm.

Nitrate reduction. Positive. Width of colour zone, 20 mm.

Growth at 37° C. None.

Liquid Media.

Saccharose synthetic solution.

Growth. Fair, star-shaped colonies at base and on sides of tube.
Surface growth. None.
Pigment. None.

Fig. 2.

Fig. 1.

MILLARD & BEELEY.—Mangel Scab—Its Cause and Histogeny (pp. 296–311).

Fig. 4.

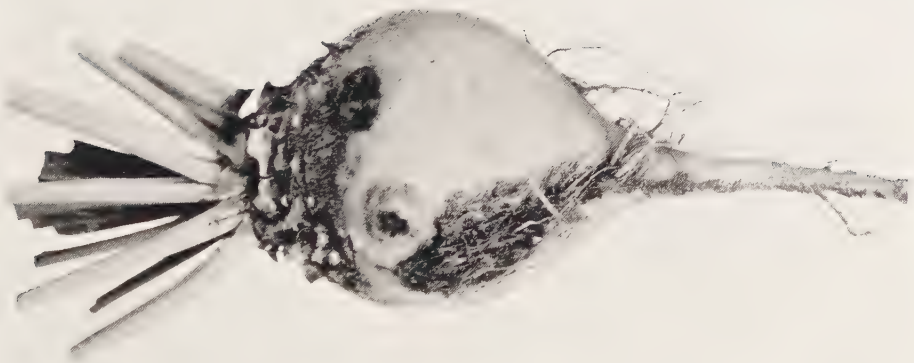


Fig. 3

MILLARD & BEELEY.—Mangel Scab—Its Cause and Histogeny (pp. 296–311).

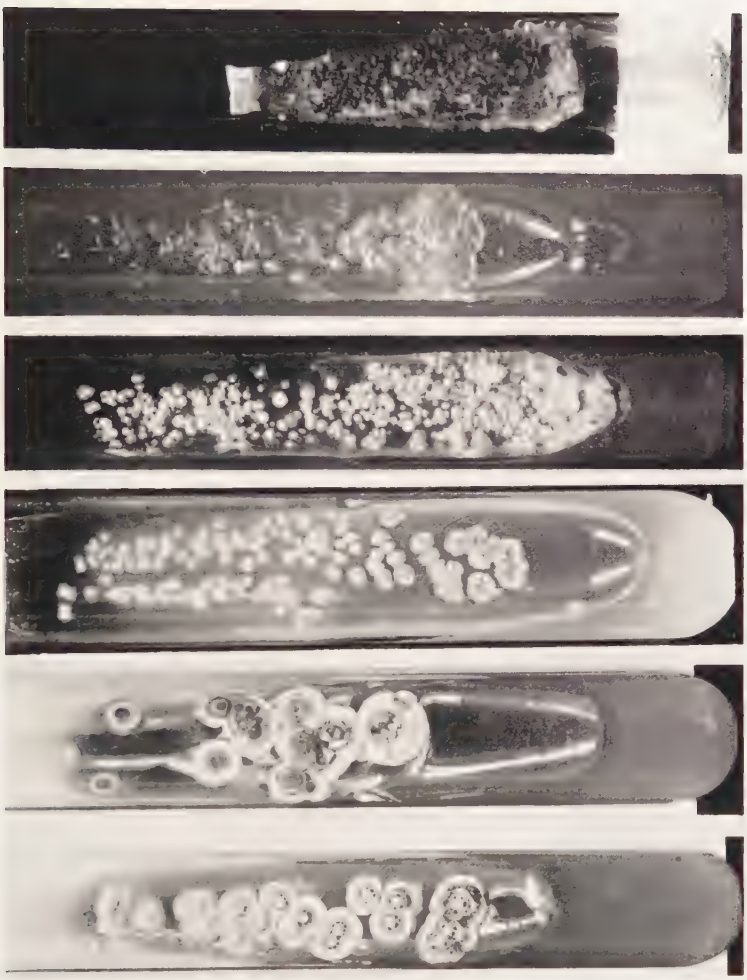


Fig. 5.

MILLARD & BEELEY.—Mangel Scab—Its Cause and Histogeny (pp. 296–311).

Fig. 6.

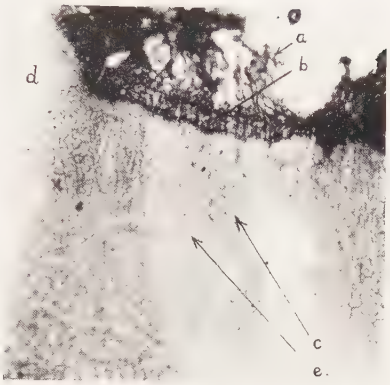


Fig. 8.

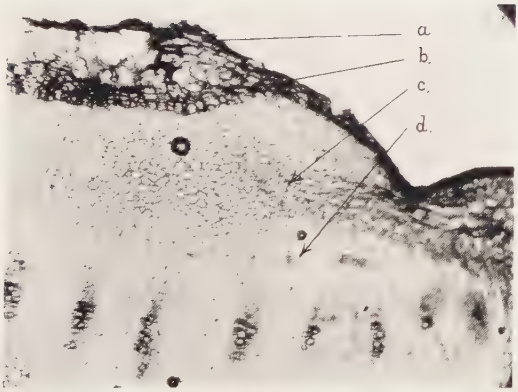


Fig. 7.

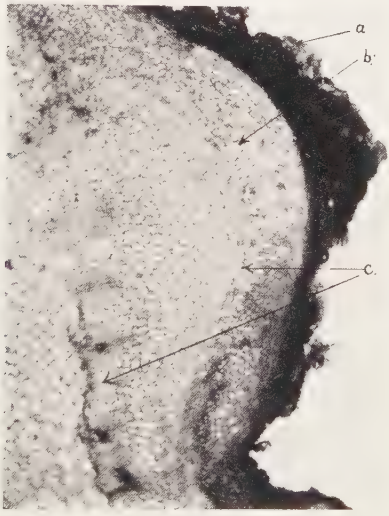


Fig. 9.

MILLARD & BEELEY.—Mangel Scab—Its Cause and Histogeny (pp. 296–311).

Glycerine synthetic solution.

Growth. Fair, dense star-shaped colonies at base and on sides of tube.

Surface growth. None.

Pigment. None.

Glucose broth.

Growth. Fair, large white spherical colonies at base.

Surface growth. None.

Pigment. None.

Brom-cresol milk.

Growth. Fair; later, good.

Aerial mycelium. None.

Coagulation. Definite clotting. Colour of clot "Water Green."

Digestion of clot. Slight.

Colour of medium. (1) "Court Grey"; (2) "Water Green."

REFERENCES.

(.) Eames and MacDaniels (1925). *Introduction to Plant Anatomy Text-Book.* McGraw-Hill Book Co.

(2) Krüger, F. (1904). Untersuchungen über den Gürtelschorf der Zuckerrüben. *Arbt. Biol. Anst.* iv, 3.

(3) Lutman and Johnson (1915). *Phytopathology,* v.

(4) Millard and Burr (1926). *Ann. Appl. Biol.* xiii, No. 4.

(5) Priestley and Bebbington, *loc. cit.*

(6) Wollenweber, H. W. (1920). *Der Kartoffelschorf,* Heft 2. Arbeiten des Forschungsinstitutes für Kartoffelbau.

EXPLANATION OF PLATES XIX—XXII.

Fig. 1. Raised scab mainly of knob type (natural infection) on Golden Tankard mangel.

Fig. 2. Raised and pitted scabs (natural infection) on Red Intermediate mangel. (*a*) Young pitted scabs.

Fig. 3. Mound scab on Yellow Globe mangel produced by inoculation with *Actinomyces* Strain 4 (Series I, Pot 7). The scab on the right has been scooped out for inoculum in reisolating the organism.

Fig. 4. Root infection produced on Red Intermediate mangel by inoculation with *Actinomyces* Strain 6 (Series I, Pot 11). Note also the swollen tissues around the bases of the rootlets on the "bulb" caused by coalescence of the scab nodules.

Fig. 5. Cultures of *Actinomyces tumuli* on (1) glycerine synthetic agar, (2) dextrose synthetic agar, (3) calcium malate glycerine agar, (4) dextrose asparagin agar, (5) nutrient potato agar, (6) potato plug.

Fig. 6. Radial section through a young mound scab showing (*a*) disorganised cortical tissue, (*b*) meristematic activity commencing in the outer layers of the pericycle.

Fig. 7. Radial section through an old mound scab showing (*a*) disorganised cortical tissue, (*b*) cork layer heavily suberised, (*c*) proliferated pericycle forming scab tissue, (*d*) outer vascular ring of the root in which the vascular elements are poorly developed.

Fig. 8. Radial section through centre of a knob scab showing (*a*) disorganised cortical tissue, (*b*) cork layer heavily suberised underlying the affected area, (*c*) proliferated pericycle, (*d*) lateral and upward outgrowth formed by the proliferating pericycle, (*e*) adventitious vascular ring formed in the scab tissue.

Fig. 9. Radial section through outer part of knob scab showing waist. (*a*) Disorganised cortical tissue, (*b*) proliferated pericycle on the outer edge of which is a cork layer 1–2 cells thick, (*c*) adventitious vascular rings formed in the scab tissue.

(*Received February 2nd,* 1927.)

A STUDY OF *HYLEMYIA (CHORTOPHILA) BRASSICAE* BOUCHÉ, THE CABBAGE ROOT FLY AND ITS PARASITES. WITH NOTES ON SOME OTHER DIPTEROUS PESTS OF CRUCIFEROUS PLANTS

By KENNETH M. SMITH, D.Sc.
(*The University, Manchester.*)

(With Plate XXIII and 10 Text-figures.)

CONTENTS.

PAGE

Introduction 312
Synonymy 313
Method of Study 313
Description and Duration of Stages 314
The Egg 314
The Larva 314
The Puparium 317
The Adult 318
Life-history and Habits 319
Number of Generations 319
Reproduction 319
Pupation 321
Hibernation 322
Feeding habits of Larva and injuries to Plants . . . 322
Food Plants 323
Other Species of Dipterous Larvae found accompanying the maggots of *H. brassicae* in Cruciferous Plants . . . 324
Parasites of *H. brassicae* 326
Summary 328
Literature Cited 329
Explanation of Plate 330

Introduction.

Although much attention has been paid in Canada and the U.S.A. to the study of the bionomics of *Hylemyia brassicae*, the cabbage root fly, it has been almost entirely neglected by the entomologists of this country and in spite of its long-continued and injurious depredations no detailed account of the fly in the British Isles exists. The writer therefore presents

this study of the cabbage root fly and its parasites in the hope that it will fill a gap in the knowledge of the injurious root-feeding insects of the country.

The writer is indebted to Mr H. Britten, Mr J. E. Collin, Mr G. T. Lyle and Dr J. Waterston for their kind assistance in the identification of many of the insects concerned in this study, to Mr James Wood for collecting material and to Miss Maud Jepson for her help in preparing the text-figures.

Synonymy.

Bezzi and Stein (7) refer to this fly as *Chortophila brassicae* Bouché, giving as synonyms *Anthomyia appendiculata* Big., *A. floccosa* Macq., *A. floralis* Fall. and *A. villipes* Zett. In 1843 Curtis (3) described an insect which he bred from cabbage roots as *Anthomyia radicum* which was probably *H. brassicae*. Gordon Hewitt (6) however describes an insect under this name which is obviously different from that described by Curtis. Seguy ((11), pp. 107–8) also gives a description of *Anthomyia radicum* under the genus *Chortophila* sub-genus *Paregle*. Slingerland (12) gives a detailed historical account of the synonymy of *H. brassicae*. He, like other American and Canadian writers, refers to this insect as *Phorbia brassicae*, *Phorbia* being synonymous with our genus *Chortophila*. In 1923 Seguy (11) placed this fly in the genus *Hylemyia*, calling it *Hylemyia brassicae* Bouché. This would appear to be the correct genus as *Hylemyia antiqua*, the onion fly, a very closely allied form, has long been placed in the same genus. To be consistent therefore with an earlier paper (13) the writer has referred to the cabbage root fly throughout this work as *Hylemyia brassicae* Bouché.

Method of Study.

The work was carried out in Lancashire and Cheshire, and the insect was studied in the field, insectary and laboratory. After some unsuccessful attempts the fly was induced to breed in captivity under the following conditions: cabbage and cauliflower plants were grown in 12-inch pots, over each was placed a glass cylinder, $2\frac{1}{2}$ ft. long with a diameter of 10 in., the top of the cylinder being closed with a piece of muslin. The insects were put into these breeding cages and were fed upon sugar and water. Under these conditions it was possible to breed a continuous series of flies throughout the season, and good opportunity was thus afforded for observing times of emergence, duration of the various periods, number of generations, etc.

Much detailed observation was also made of the insect in the field upon the cruciferous crops of the counties and upon a large plot of cabbages and cauliflowers grown for this purpose.

Description and Duration of Stages.

The Egg.

The egg is white and easily visible. In shape it is cylindrical, one end being pointed, the other somewhat truncated: in length it measures about 1 mm. and is less than half that in width. The shell or chorion is sculptured into longitudinal ridges; down one side of the egg for three-quarters of its length run two well-marked parallel sutures. Between these sutures is a somewhat depressed area in which the ridged sculpturing is less pronounced than that in the rest of the chorion. When the egg hatches, the smoother area between the sutures is pushed out, the larva emerging at the truncated end where the space between the sutures is widest.

Duration of Egg Stage. From a number of observations in field and laboratory, it would appear that from four to five days is the normal duration of the egg stage. A fly was observed ovipositing on young cabbage plants in the field on May 31st. After some half dozen eggs had been laid, they were collected and placed under observation in the laboratory. They hatched in the early hours of the morning of June 4th. Similar observations a week later also gave an average duration of the egg period as four days. This length of time however may vary as in some cases, especially the second generation, the length of the egg period was reduced to three days.

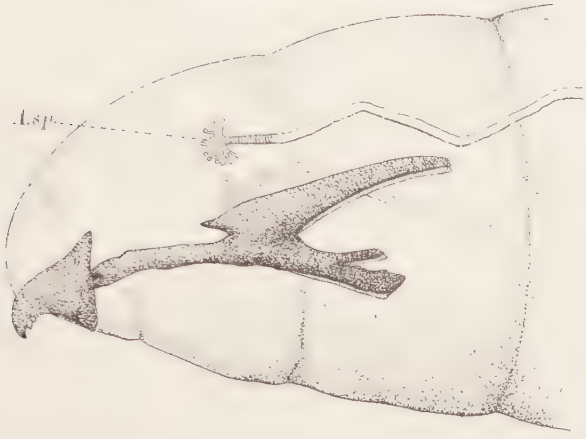
The Larva.

First Instar. On hatching from the egg the young maggot measures slightly over 1 mm. in length. It is curved, white in colour and the segments and tubercles are distinct. In this stage there are no anterior spiracles and the buccal skeleton differs slightly in appearance from that of the second and third instars. Text-fig. 1 is a drawing of the first-stage larva.

Second Instar. The second-stage larva measures from 2 to 4 mm. in length. Both anterior and posterior spiracles are now present but the posterior spiracles differ from those of the third instar in having only two openings in each spiracle instead of three. *Vide* Text-fig. 4 B.

Third Instar. The third-stage larva varies from 2 to 8 mm. in length and from 1 to 2 mm. in diameter. It is white, tapering at one end and

Fig. 1. First-stage larva of *H. brassicae*.



A.sp. = anterior spiracles.

Fig. 2. Anterior segments of mature larva of *H. brassicae*.

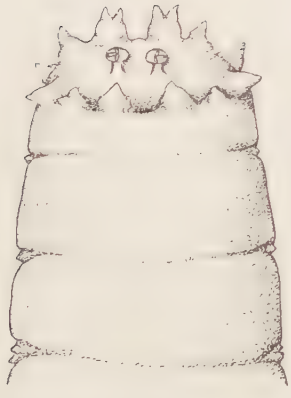


Fig. 3. Posterior segments of mature larva showing arrangement of tubercles.

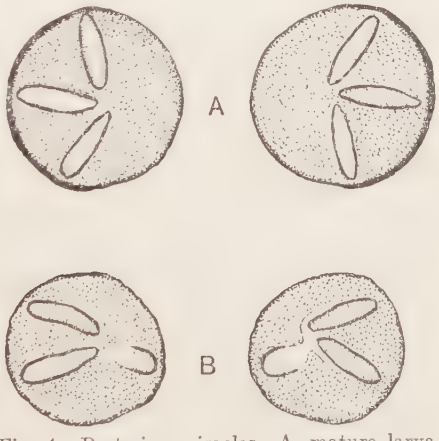


Fig. 4. Posterior spiracles. A, mature larva. B, second-stage larva. In B the third spiracular opening is in process of being formed.

truncated at the other, the caudal end. The integument is somewhat smooth and shining; this fact constitutes a point of difference between it and some other maggots of similar habits. At the anterior end there is a strong buccal skeleton, the lateral plate being forked, *vide* Text-fig. 2. The anterior spiracles consist of a pair of fan-shaped organs situated laterally containing from 11 to 13 processes. The number of these processes is not constant even in individual larvae, occasionally 11 being present on one side and 13 on the other. The number of these processes varies between 10 and 13 in the mature larva and possibly rather fewer in the second-stage larva. There are two pairs of tubercles surrounding the buccal opening. At the posterior end there is a caudal corona consisting of six pairs of tubercles, while a seventh pair is situated ventrally near the anus. The outstanding feature of this corona is the pair of bifid or forked tubercles on the ventral rim of the last segment, these forked tubercles are important in the identification of this larva, cf. Figs. 7–10. The arrangement of the caudal tubercles is illustrated in Text-fig. 3. In the centre of the caudal disc arise the knob-like posterior spiracles which are the external openings of the tracheae, they are brownish yellow in colour, heavily chitinised and each one opens with three slits. Text-fig. 4 is a drawing of these posterior spiracles showing the slit-like openings.

Duration of Larval Life. In 12 series of larvae of the first and second generation under observation in the insectary, for the duration of the larval stage, the periods varied between 19 and 25 days with an average of 23 days. Observations on the earlier maggots of the third generation gave an approximately similar period but a considerable proportion of the later maggots of this generation took much longer to pupate, some existing in the larval condition for as long as 35 days.

From observations made the writer considers it possible that some larvae of the third generation may hibernate as such. A number of maggots hatching from a batch of eggs on September 4th fed normally till the 21st of the month, being by that time nearly full grown. A spell of cold weather then set in and the maggots ceased feeding and appeared to rest in a state of suspended animation. One larva was removed to the greater warmth of the laboratory where it immediately pupated, a week later a second was removed under similar conditions with the same result. Meanwhile of the remaining larvae, each one had hollowed out a small cell in the cabbage root where it remained till the end of the first week in October, when the temperature rose and the larvae commenced to pupate, the last one transforming on October 11th. It is of

interest to note that examination of these quiescent larvae showed them to be of unusual size and to be provided with a large quantity of fat as if in preparation for a long rest. It is thus possible that the maggots found in cabbage roots during November and December are hibernating, although all maggots in the insectary had pupated by October 25th.

The Puparium.

The puparium is a seed-like object rounded at both ends. It is very variable both in size and colour, the more normal specimen measuring 6–7 mm. long by 3 mm. broad at the centre and being of a reddish brown colour. Often however puparia are found of approximately half this measurement, the variation in size being probably governed by the amount of food available for the larva. The colour also varies from deep red to light brown. The integument of the puparium consists of the hardened skin of the larva, and some larval characteristics are still visible in the shape of the bifid tubercles at the posterior end of the puparium and the spiracles at the anterior end. Text-fig. 5 is a drawing of the puparium.

Fig. 5. Puparium of *H. brassicae*. *B.T.* Bifid tubercles of larva.

Duration of the Pupal Stage. That the length of the pupal period is very variable is shown by the following observations. Out of a total of 51 first-generation pupae, 29 gave an average duration of 15 days, the remaining 22 gave an average period of 35 days, the longest time of this generation being from June 29th to September 6th, *i.e.* 69 days, and the shortest period from July 8th to July 19th, *i.e.* 11 days. From a similar series of observations on the second-generation pupae the average period was 15 days, the longest period being 18 days and the shortest 7 days. A considerable proportion of this brood did not hatch

until the following year. The third-generation pupae hibernated *in toto*, giving rise to adults in the following spring.

The Adult.

Male Fly. The size is variable, averaging 6 mm. It is dark grey in colour with lighter markings. The eyes are sub-contiguous and occupy the greater portion of the head. Dorsally the thorax possesses three well-marked longitudinal bands, the centre band being continued over the abdomen which is rather small and tapering. The squamae are large, wing veins brown. The legs are black with a cluster of bristles on the hind femora, while the whole fly is thickly covered with black bristles.

Female Fly. Eyes widely separated, body much lighter in colour than the foregoing, abdomen broader and more pointed, legs brownish grey without the tuft of bristles characteristic of the male. As a whole the female fly is less bristly.

Duration of Life of Adult. When fed on sugar and water the flies lived in captivity for considerable periods; both sexes lived for as long as 35 days, and in one or two cases even longer. Duration of life in the male ranged from 9 to 35 days and in the female 14 to 38 days. If no food was provided the male flies commenced to die after 3 days and the females after 4 or 5 days. It may be of some interest to note in this connection the extraordinary degree to which the flies in captivity, especially the females, would gorge themselves upon the sugar and water supplied to them. The writer had under observation two females which had become enlarged by this procedure to almost twice their normal size. The abdomen was distended until it was nearly circular, the proboscis was extended and could not be withdrawn, and the antennae were fixed in an abnormal upright position giving a horned appearance to the insects. These two flies were removed, placed in an empty glass vessel, and kept under observation. After 2 or 3 days the swelling began to subside, until, a week after their transfer to the empty jar, the flies had resumed their normal appearance, apparently little the worse for their adventure. This desire for sugar might be profitably employed in the use of a poison bait on the lines of that so often recommended for the onion fly.

Total Length of Life-cycle. From observations made in the insectary upon flies bred in captivity, the average length of the life-cycle from egg to adult would appear to be 46 days, with a minimum observed period of 37 days and a maximum of 53 days.

Life-history and Habits.

Number of Generations.

There are three generations of *H. brassicae* in the year. By the method of continuous breeding adopted in this study, it was possible to observe the number of broods per season with considerable accuracy. As in the case of *H. antiqua*, the onion fly, a closely allied insect, there is no sharp line of demarcation between each brood, and larvae of the second generation were often still feeding at the same time as larvae of the third brood. Similarly some second-generation adults were still hatching during the period of maximum emergence of the third-generation adults. The flies of the first generation hatching from the overwintering puparia begin to appear about the end of the second week in May, the earliest record for this district being May 14th.

In the present year (1926) females were first observed ovipositing on May 31st. The first fly of the second generation hatched in the insectary on June 29th, the time of maximum emergence being from July 8th to July 18th, although flies of the second brood continued to emerge at infrequent intervals throughout August, the last to emerge appearing on September 6th, after having pupated on June 29th. The first adults of the third generation commenced hatching on July 27th, the period of maximum emergence being from August 7th to 13th. The third generation continued to emerge throughout August up to the second week in September, the last adult female observed being collected in the field on September 29th. This fly died almost immediately, all the eggs having been deposited as was revealed by an examination of the insect's oviducts. It is of interest to note that a considerable proportion of the second-generation pupae which normally should give rise to third-brood flies did not hatch at all in the year of pupation but remained in the pupal condition until the following year. This same phenomenon is to be found in the case of other root-feeding species.

Reproduction.

Oviposition. Prior to laying her eggs the female fly examines the soil surrounding the stalk of the cabbage or other cruciferous plant, prodding and testing the cracks with extended ovipositor. As a rule the eggs are deposited on the stem at, or just below, the soil level. Often however they are placed in the soil immediately surrounding the stalk or in the cavity between stalk and soil. The number of eggs deposited

at one time varies from two or three up to a dozen or more; one female fly under observation deposited six before moving away to fresh plants. These remarks apply chiefly to the first two generations of flies; in con-

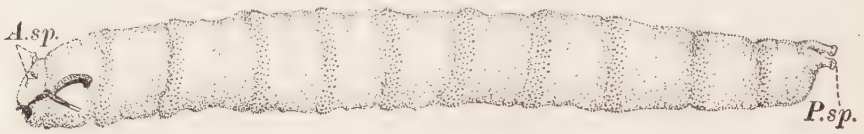


Fig. 6. Larva of *Phytomyza rufipes* Meig. *A.sp.*, anterior spiracles. *P.sp.*, posterior spiracles.

Fig. 7. Dipterous larva of unknown species from cabbage root. *B.T.*, one bifid tubercle.

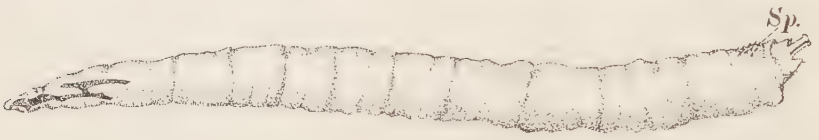


Fig. 8. Dipterous larva from cabbage stem. Probably a feeder on decaying matter. *Sp.*, spines.

Fig. 9. Dipterous larva from cabbage stem. Probably a feeder on decaying matter.

Fig. 10. Larva of *H. brassicae* for comparison with Figs. 6–9. *A.sp.*, anterior spiracles. *P.sp.*, posterior spiracles. *B.Tt.*, two bifid tubercles.

sidering the egg-laying habits of the third generation and especially the latterly hatched flies of this brood, a somewhat different habit is revealed. On the three days September 15th–17th the writer collected

150 eggs from the following situations—deposited singly or in pairs at regular intervals along the midrib and larger veins of cauliflower and cabbage leaves, in fair numbers on the cut surface of cauliflower stems where the head had been removed, and also inside the cauliflower head, one or two eggs on each stalk of the inflorescence. These eggs were collected and kept under observation in the laboratory in order that their identity might be definitely established although external examination revealed no difference from the normal eggs of *H. brassicae*. Of these eggs a considerable proportion hatched into the cabbage-root maggot, there were also however larvae of different species of Anthomyids (*vide* Text-figs. 6–10), which latter had probably been induced to oviposit by the beginnings of decay in the plants in question. If further proof be needed, the presence of *H. brassicae* larvae in the midrib and large veins of cauliflower and cabbage leaves in late September seems indicative of a difference between the egg-laying habits of the third generation and of the two preceding it. The fact that in the autumn the stems of Brassicas are hard and woody is probably sufficient reason for a change in the place of egg deposition. Theobald (15) describes cases of *H. brassicae* larvae in the midribs of cauliflower and cabbage leaves and Gibson and Treherne ((5), p. 24) also mention that they have observed the eggs of this fly deposited on the fleshy leaf stalks, though apparently they are of opinion that this is a rare occurrence.

Pre-oviposition Period. The following observations were made on the time elapsing between date of hatching and date of oviposition.

Adults emerged	July	9th,	eggs deposited	July	18th
,,	,,	10th,	,,	,,	19th
,,	Aug.	8th,	,,	Aug.	15th
,,	,,	15th,	,,	,,	21st–22nd

Another batch of adults hatching on August 18th was noticed *in coitu* on August 26th, the eggs being deposited 24 hours later, *i.e.* August 27th. Females dissected 5 days after emergence were still immature but others dissected 8 days after hatching possessed some mature ova. From these observations the pre-oviposition period would appear to last between 7 and 8 days. Gibson and Treherne ((5), p. 27) put the period at 6 days, and Schoene (10) from 3 to 5 days.

Pupation.

Pupation takes place in the soil surrounding the roots of the host plant, though occasionally puparia are found adhering to the root itself. In an examination in the field of a large number of infested plants the

majority of puparia were found at a depth of 3 to 5 in., while some, particularly of the later broods, were as deep as 8 or 9 in. Occasionally the larvae migrate laterally and puparia may thus be found near the surface of the soil 3 or 4 in. away from the plant. The adult insect seems able to emerge through a considerable depth of soil; in the laboratory pupae placed at the bottom of tall glass cylinders gave rise to flies which emerged easily through the 7 in. of soil superimposed.

Hibernation.

The most usual method of hibernation is undoubtedly in the pupal stage. There is however certain evidence that the larvae may also hibernate. The writer has observed on several occasions large larvae with an unusual accumulation of fat in cabbage roots during October. These larvae did not appear to be feeding but remained motionless in small cells in the tissue. There is no doubt that a low temperature influences the larval development, and even if the larvae do not actually hibernate, they are capable of remaining in a state of suspended animation for considerable periods before finally pupating. Miles (9) records the larvae feeding in the heads of cauliflowers as late as November. These larvae had probably developed from eggs deposited in the heads of the cauliflowers by late-hatched flies of the third generation. Gibson and Treherne (5) and Schoene (10) also state that they have found larvae throughout the winter. It is thus reasonable to postulate that a small percentage of larvae do hibernate but in the writer's opinion they are capable of surviving only mild winters.

As regards hibernation in the adult form, evidence on this point is very scanty. Various writers have at different times put forward the view that the adult flies do survive the winter, but up to date no convincing evidence has been offered on this point. There is no doubt that the pupal stage is the usual condition for hibernation.

Feeding Habits of Larva and Injuries to Plants.

On hatching from the egg the young larva as a rule makes for the root just below soil level, bores into the cortex and tunnels up and down. A plant is usually able by throwing out adventitious rootlets to withstand attack by a few maggots only, but where a dozen or more are present the whole root becomes hollowed out and the plant quickly dies. Although the name "root maggot" has been applied to the larvae of *H. brassicae*, the root is by no means the only, though possibly the most common, habitat of these maggots. The writer has had experience of a

field of cauliflowers which, although apparently otherwise healthy, yet were entirely blind. An examination of these plants revealed the presence of *H. brassicae* larvae in large numbers feeding at the apex of the stem and thus preventing the formation of the head. The roots however of these cauliflowers were quite healthy and untouched by the larvae. This habit of feeding in the heads of cauliflowers is more typical of the late brood, as is revealed by the oviposition habits of the third generation. The larvae have also been observed feeding in the growing points of turnips. In autumn, when the roots of cauliflowers and cabbages are hard and woody, the later larvae of the third brood may sometimes be found tunnelling in the midrib and large veins of *Brassica* leaves and also at the leaf bases. In October the writer has found the larvae to be more common in turnips than in Brassicas.

Injury to host plant depends therefore on the place of attack and number of maggots attacking. From an examination in the field of some 200 infested cabbage plants, the number of maggots in the roots varied from 9 to 46. Gibson and Treherne ((5), p. 21) have found up to 127 maggots in a single plant, but such a large number is unusual in this country. The symptoms of maggot attack at the roots of Brassicas are characteristic. The plant first of all becomes limp and flaccid, the leaves turning bluish, later becoming yellow with complete collapse in a bad attack.

H. brassicae larvae may therefore be looked for in the following situations: (*a*) In the roots of Cruciferous plants, the most usual mode of attack. (*b*) In the growing point of turnips and of cauliflowers before the head is formed. (*c*) In the inflorescence of cauliflowers. (*d*) In the midrib and larger leaf veins of Brassicas. This last mode of attack should not be confused with that by another species of Dipterous maggot (*vide* Text-fig. 6) which normally tunnels in the midrib of *Brassica* leaves.

Food Plants.

The food plants of *H. brassicae* seem to be confined almost wholly to the Cruciferae. The larvae occur commonly in cabbage, cauliflower, brussels sprouts, kale, radish, turnips and swedes and the writer also has had experience of their feeding at the roots of stocks. Leaflet No. 122 of the Ministry of Agriculture gives several wild Cruciferous plants as hosts of this maggot, such as shepherd's purse, charlock, hedge mustard, etc. Chittenden(2) records celery as an occasional host plant and Gibson and Treherne ((5), p. 20) give instances of damage to beet. As a rule however Cruciferous plants only are attacked. It is doubtful whether

there is much preference for attack within the varieties of Brassicas; the fly chooses young and tender plants of cabbage, cauliflower, or brussels sprouts apparently indiscriminately. A field of young sprout seedlings growing alongside a field of young cabbage was observed to be quite as heavily infested as the latter. Probably however, when the sprouts are more mature, infection is less common than with other Brassicas.

Other Species of Dipterous Larvae found accompanying the Maggots of H. brassicae *in Cruciferous Plants.*

It does not lie within the scope of the present paper to give a detailed description of the other Dipterous pests of Cruciferous plants. Such description would entail a considerable amount of further study on a subject concerning which little is known. Nevertheless the writer considers it worth while to put on record the following facts concerning four other species of Dipterous larvae which were found associated with *H. brassicae* in Cruciferous plants.

Phytomyza rufipes Meig. (J. E. Collin det.). The larva of this insect was found in the upper part of the root of Brassicas and in the leaf bases; the largest number however was found tunnelling in the midribs of cauliflower and cabbage leaves. It is a smooth shining maggot about 6 mm. long when full grown. Each segment is strongly differentiated in the mature larva by a concentric ring of small spines surmounting each segmental swelling. The anterior spiracles stand well out from the body, and the posterior spiracles are directed downwards and backwards, forming a distinctive character by which the larva is easily identified. There is a complete absence of tubercles. These larvae were found causing considerable damage to the leaves of cauliflowers in autumn, the blackened borings in the midrib being distinctly visible from the exterior. More serious damage is occasionally done to young cauliflowers through these maggots boring in the head and causing blindness. Text-fig. 6 is a drawing of this larva. The puparium is to be found in large numbers in the soil in company with those of *H. brassicae*. It is seed-like in appearance, of a light brown colour and measures about 4 mm. in length. Most of the larval characteristics are still visible, particularly the anterior and posterior spiracles, and it is also possible to make out the segmental rings of larval spines. The adult is a small fly measuring 7 mm. across the wings, antennae very short and broad, yellow in the male, arista long and bare, head brownish with some white markings round eyes, bristles sparse. There is a small black raised area containing

spines situated between the eyes, legs and wing veins brown, thorax greyish with a number of long bristles sparsely set. Four pairs of long spines form two ill-defined rows down the centre of the dorsum, scutellum large, halteres yellowish white, abdomen greyish, thickly set with bristles. Female with black antennae, except for the extreme edge, which is yellow.

The eggs are inserted in the tissue of the leaf blade, the place of oviposition being marked by a small whitish ring. The larvae live mostly in the midrib and leaf bases, occasionally being found in the stem and growing points of young plants. Pupation takes place mostly in the soil, though puparia have occasionally been found in the leaf veins. There appear to be three or four generations in the season, adult flies being plentiful from May to October. The larva is heavily parasitised by *Dacnusa stramineipes* Halid. (Plate XXIII, fig. 4) which also parasitises the larva of *H. brassicae*. Theobald(15) refers to a fly which he bred from Crucifers as *Phytomyza flavicornis* Meig., this is probably the same insect. The second type of larva sometimes found associated with *H. brassicae* occurred on two occasions in the root of Brassicas. This larva (Fig. 7) resembles the cabbage-root maggot somewhat, but there is a considerable difference in the character of the larval skin. In *H. brassicae* it is smooth while that of the species under observation is muricate or shagreened, being entirely covered with minute spines. The caudal tubercles also differ in their arrangement, there being only one bifid tubercle. The writer failed to breed the flies from these larvae and so cannot state the species. Keilin, however(8), records finding larvae of a different type feeding in the roots of Crucifers; these gave rise to a new species of fly which was named *Chortophila pilipyga* Villeneuve, and later re-named by Seguy(11) as *Hylemyia pilipyga*. It is possible that these rough-skinned larvae may be the maggots of the same insect.

The other two types of larvae are illustrated in Text-figs. 8, 9.

These two species of larvae were only present in cabbage stems in which there was a certain amount of decay and the writer considers them to have been living mainly upon decaying tissue and not to be of true phytophagous habit. The species illustrated in Text-fig. 8 was a slender and tapering larva with a rough muricate skin; it possessed a patch of spines (*sp.*) situate dorsally near the posterior end. The other species, Text-fig. 9, was less slender and tapering and possessed a smoother skin. The arrangement of the caudal tubercles in each species was characteristic and is illustrated in the text-figures. A line drawing of the larva of *H. brassicae* (Text-fig. 10) is also given for comparison with the other types.

Parasites of H. brassicae.

In the course of this study the following parasites were bred from *H. brassicae*:

COLEOPTERA. Staphylinidae.

Aleochara bilineata Gyll. The larva of this beetle is predaceous upon the pupae of the cabbage root fly. According to Wadsworth (17) the first-stage beetle larva bores its way into the puparium of the Dipterous host and completes its metamorphosis inside, feeding upon the pupa, emerging finally as the adult beetle. The writer bred large numbers of this beetle from the pupae of *H. brassicae*, and there is no doubt that it constitutes a considerable check upon the development of the fly. Fowler (4) gives a description of this beetle and the closely allied species *Aleochara bipustulatus* (*nitida* Grav.). From the puparia containing this beetle larva, the writer bred a Hymenopterous insect which was parasitising *A. bilineata*. This insect has been determined by Waterston as the Proctrotrupid, *Exallonyx ligatus* Nees. Plate XXIII, fig. 1, shows this Proctrotrupid, the beetle larva from which the parasite was bred, and the ruptured pupal case of *H. brassicae*, in which both these insects were contained. The empty pupal case of *Exallonyx ligatus* still attached to the beetle larva may also be seen.

Aleochara bipustulatus (*nitida* Grav.). The writer found this smaller Staphylinid often associated with *H. brassicae* puparia at the roots of Brassicas. Although none were bred from puparia under observation, it is likely that this insect also is predaceous upon the pupae of *H. brassicae*.

HYMENOPTERA. Cynipidae.

Cothonaspis rapae Westw. This Cynipid is by far the commonest of all the parasites affecting *H. brassicae* and was bred in very large numbers. Over 30 per cent. of the second-generation pupae were parasitised by this insect. There appear to be two broods in the season, early in June a female was observed ovipositing in first-generation larvae. The second-generation adults commenced hatching on July 26th and continued to hatch during August and September. The times of emergence of the Cynipid were very variable, the parasites hatching from both first and second *H. brassicae* pupae as late as August 31st. The date of the last emergence of *Cothonaspis rapae* was October 4th. It was observed that the pupal period of this Cynipid seemed to last a considerable time, and this may further indicate that there are two generations only. Parasitised larvae of *H. brassicae* pupating on June 24th

gave rise to the parasite on July 29th, 30th and August 3rd. Plate XXIII, figs. 2 and 3, are photographs of *Cothonaspis rapae* Westw.

The next Hymenopterous parasite is a species of Braconid, *Dacnusa stramineipes* Halid. This insect chiefly parasitises the Phytomyzid larva (*P. rufipes*) but the writer bred it also on two occasions from the pupae of *H. brassicae*. Plate XXIII, fig. 4, is a photograph of this parasite.

In addition to the Hymenopterous parasites of *H. brassicae* here described, Wadsworth(18) records two further insects—the Ichneumons *Phygadeuon fumator* Grav. and *Atractodes tenebricosus* Grav. (*vestalis* Hal.). The writer has not succeeded in breeding these latter from pupae of *H. brassicae*.

DIPTERA. Anthomyidae.

Phaonia trimaculata Bouché. The larvae of this large Anthomyid fly occur in the roots of Cruciferous plants in association with the larvae of *H. brassicae* upon which they feed. These carnivorous larvae may be distinguished from the root maggots by their larger size and by the absence of the well-marked caudal tubercles. The pupae of *Phaonia* were also found by the writer together with those of *H. brassicae* in the soil surrounding the plant, distinguishable from the latter by their larger size. Bouché(1) first described this fly as *Anthomyia trimaculata*, and Wadsworth(17) recorded it for the first time in this country in 1915. Keilin(8) also has a note upon the carnivorous character of the larvae. The fly is light grey in colour with four black bands on the dorsum, legs black, and abdomen with a black dorsal band.

The last insect associated with *H. brassicae* to be described is the Tachinid fly *Onesia agilis* Meig. (*vespillo* Rdi.) and its precise connection with the cabbage root maggot has not been determined.

The pupae of this fly were found on several occasions in company with those of *H. brassicae*. There seem to be two possible explanations concerning the activities of this fly, either, like the foregoing *Phaonia*, the larvae are carnivorous and prey upon the cabbage root maggot or they are, like many Tachinid larvae, feeders in decaying matter and are thus only secondary to the damage caused by *H. brassicae*.

As however there seems a definite association of this Tachinid with the cabbage root fly, the writer has considered it worth while to put the fact on record.

Plate XXIII, fig. 5, figures an interesting case of parasitism of the adult fly by some micro-organism, the exact nature of which has not yet been

determined. In the field a number of Anthomyid flies (not all *H. brassicae*) were observed to have an abnormal appearance about the abdomen. On capturing and examining the flies, they were found to be in a most interesting and unusual condition. Dorsally the abdomen presented a slightly rounded and swollen aspect, while ventrally there was a very large circular opening or crater with rounded lips leading into a cavity which occupied the greater part of the interior of the abdomen. In one or two cases there existed two of these crater-like orifices side by side in the abdomen of the same fly. There were no external signs of the disease visible in the fly other than the slight swelling referred to, and the crater-like opening or openings. It seems a remarkable thing that the flies so attacked were able to live an apparently normal life in spite of the fact that the abdomen was entirely occupied by a cancerous growth with its external orifice. Sections of these diseased flies revealed a cyst-like structure with an internal cavity opening to the exterior by the ventral orifice already referred to. Microscopical examination showed the presence of a micro-organism which, though unlike the parasitic *Empusa* in many ways, yet possessed some characteristics of that organism. At the same time minute spore-like bodies are present which cannot be attributed to *Empusa* and appear more characteristic of some Protozoan group (14).

Summary.

A detailed account is given of the life history and parasites of *Hylemyia* (*Chortophila*) *brassicae*, the cabbage root fly. The various stages are described and figured. The egg is deposited in the vicinity of the roots of Cruciferous plants, in the heads of cauliflowers, and in autumn on the leaves and cut stem of Brassicas. The period of the egg stage lasts from 3 to 5 days, the larva when full grown is smooth, white in colour, and possesses a caudal corona of well-defined tubercles, one pair of which is bifurcate and serves as a means of identification. The maggots feed in the roots of Cruciferous plants, or in the heads of cauliflowers and turnips, while late in the autumn they may occasionally be found tunnelling in the midrib of *Brassica* leaves. The larval period lasts from 19 to 25 days. The puparium is reddish brown in colour and measures about 7 mm. in length. The length of the pupal stage is very variable and may occupy from an average of 16 days to 69 days. Pupation takes place in the soil in proximity to the plant. The adult is a dark grey insect somewhat resembling the house fly but is more bristly.

There are three generations in the season; as a general rule hibernation is in the soil in the pupal stage.

Four other types of larvae found associated with those of *H. brassicae* in Cruciferous plants are described, the most important being *Phytomyza rufipes* Meig.

A detailed description of the parasites of *H. brassicae* is given; these are the larva of the Staphylinid beetle *Aleochara bilineata* Gyll., the Cynipid *Cothonaspis rapae* Westw., the Braconid *Dacnusa stramineipes* Halid. and the Anthomyid fly *Phaonia trimaculata* Bouché, the larvae of which are carnivorous and feed upon *H. brassicae* larvae. There are also described a Proctrotrupid, *Exallonyx ligatus* Nees., which is parasitic upon the larva of *Aleochara bilineata* Gyll. and a Tachinid fly, *Onesia agilis* Meig., which is associated with *H. brassicae*, although the exact relationship has not been determined.

An unusual form of parasitism of the adult fly by some kind of micro-organism is also figured and briefly described.

LITERATURE CITED.

(1) Bouché, P. (1834). *Naturgeschichte der Insekten.*

(2) Chittenden, F. H. (1902). *U.S. Dept. of Agric. Div. of Entom. Bull.* 33, N.S., p. 80.

(3) Curtis, J. (1860). *Farm Insects*, p. 141.

(4) Fowler, Canon (1888). *British Coleoptera.* Vol. II. Staphylinidae, pp. 18–19.

(5) Gibson, A. and Treherne, R. C. (1916). The Cabbage Root Maggot and its Control in Canada. *Dept. of Agric. Entom. Branch. Bull.* No. 12. Ottawa.

(6) Gordon Hewitt, C. (1907–8). *Journal Econ. Biology*, II, 56.

(7) Bezzi, M. and Stein, P. *Katalog. der Palaarktischen Dipteren*, III, 1907, 712.

(8) Keilin, D. (1917). *Parasitology*, IX, 440.

(9) Miles, H. W. (1923). *Ann. Rept. Kirton Agric. Inst.* p. 48.

(10) Schoene, W. J. (March, 1916). The Cabbage Root Maggot. Its Biology and Control. *New York Agric. Exp. Station Bull.* No. 419.

(11) Seguy, E. (1923). *Faune de France. Diptères Anthomyides*, p. 91. Paris.

(12) Slingerland, M. V. (1894). The Cabbage Root Maggot. *Cornell Univ. Agric. Exp. Station Bull.* 78, pp. 572–577.

(13) Smith, Kenneth M. (Nov. 1922). A Study of the Life-History of the Onion Fly (*Hylemyia antiqua* Meig.). *Ann. App. Biol.* IX, Nos. 3 and 4.

(14) —— (1927). An Unusual Form of Parasitism of an Anthomyid Fly. *Parasitology*, XIX, No. 2.

(15) Theobald, F. V. (1905–6). *Rept. Econ. Zool. S.E. Agric. Coll.*

(16) —— (1910). *Ibid.* p. 100.

(17) Wadsworth, J. T. (June 1915). On the Life-History of *Aleochara bilineata* Gyll. A Staphylinid Parasite of *Chortophila brassicae* Bouché. *Journ. Econ. Biol.* X, Nos. 1 and 2.

(18) —— (1915). *Entom. Monthly Magazine*, p. 142.

(19) —— (July 1915). Notes on Some Hymenopterous Parasites. *Ann. App. Biol.* II, Nos. 2 and 3, 158–161.

EXPLANATION OF PLATE XXIII.

Fig. 1. *Exallonyx ligatus* Nees. A Proctrotrypid, parasitic upon the larva of *A. bilineata*. In the figure the Proctrotrypid may be seen on the left, the parasitised beetle larva in the centre, and the pupal case of *H. brassicae* from which both insects have come is seen on the right. Note the empty pupal case of *E. ligatus* attached to the tail of the dead *Aleochara* larva.

Fig. 2. The Cynipid parasite *Cothonaspis rapae* Westw. Male. ×5.

Fig. 3. *Cothonaspis rapae* Westw. Female. ×5.

Fig. 4. The Braconid parasite *Dacnusa stramineipes* Halid. ×5.

Fig. 5. Adult female of *H. brassicae* showing attack by a micro-organism. The large crater-like opening of the internal cyst is plainly shown. ×5.

(*Received November 19th*, 1926.)

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

SMITH.—A Study of *Hylemyia brassicae*, the Cabbage Root Fly (pp. 312–330).

STUDIES ON CONTACT INSECTICIDES

PART VI. THE INSECTICIDAL ACTION OF THE FATTY ACIDS, THEIR METHYL ESTERS AND SODIUM AND AMMONIUM SALTS

By F. TATTERSFIELD and C. T. GIMINGHAM.
(*Insecticides and Fungicides Department, Rothamsted Experimental Station, Harpenden.*)

(With 9 Text-figures.)

TABLE OF CONTENTS.

PAGE

Introduction 331
Experiments with *Aphis rumicis* 333
The Fatty Acids 334
The Sodium and Ammonium Salts of the Fatty Acids . . 339
The Methyl Esters of the Fatty Acids 342
Experiments with Eggs of *Selenia tetralunaria* . . . 346
Molecular Weight in Relation to Insecticidal Action . . 348
Some Physical Properties in Relation to Insecticidal Action 348
Physical State 349
Volatility 349
Dissociation Constants 350
Partition Coefficients 351
Surface Tension 356
Summary 357
Literature Cited 358

Introduction.

It has been a common practice for many years to incorporate solutions of the salts of the higher fatty acids with insecticide and fungicide spray fluids, mainly with the object of increasing their wetting and spreading powers. The potash soaps of the liquid fatty acids such as oleic are invariably used, as their solutions are fluid at ordinary temperatures. In recent years however in the case of certain basic insecticides such as nicotine, the fatty acid has been combined directly with the organic base. It is generally known that, apart from their effect

upon the physical properties of the spray, these soap solutions themselves are toxic to insects.

The insecticidal properties of some of the fatty acids and soaps have recently been investigated by Siegler and Popenoe (1, 2). Their experiments were made on several species of aphids with the free acids, and with acid, neutral and alkaline soaps. The free acids were emulsified with a solution of glue, or, when used with water alone, by means of a colloid mill, and sprayed on to the insects with a small atomiser. They found that whereas the lower acids showed no great toxicity to aphids, caproic acid showed "marked toxicity," and caprylic acid killed 90 per cent. at a dilution of 1 in 500, with only slightly decreased toxicity at 1 in 1000. Capric acid killed 99 per cent. and lauric acid 92 per cent. at 1 in 1200. Myristic acid was less toxic. The figures do not all apply to the same species of aphid. Rapid and complete paralysis of the insects was noted and the hypothesis is put forward that "the fatty acids in a crystalloid state penetrate the body walls and tracheae, thus gaining immediate haemolytic action on the haemolymph and body cells" (2). Further, these investigators consider that the toxicity of soaps is chiefly due to the action of free fatty acid liberated by hydrolysis.

Siegler and Popenoe have, so far as we are aware, published only brief accounts of their experiments, without any detailed figures. The fatty acid series appears particularly suitable for the study of change of toxicity with increase of molecular complexity, for each member differs from the next by one CH_2 group only, and all except formic acid contain a chemically reactive carboxyl group united to a comparatively unreactive hydrocarbon chain. This series of acids up to stearic was therefore included in our survey of the insecticidal action of various groups of organic compounds.

The data presented here indicate that within a wide range there is a steady increase in toxic action, as the series is ascended. There is moreover a gradation in certain physical properties, and the question arises whether the change in toxic action is not determined largely by one or more of these dependent properties rather than directly by the molecular complexity of a relatively inert hydrocarbon chain. A preliminary attempt is therefore made to trace out relationships between certain physical factors and the toxic action among these acids. The carboxyl group is readily esterified and saponified, giving compounds with new sets of properties, and the methyl and benzyl esters and the sodium and ammonium salts of a number of these acids have been investigated. In each case interesting quantitative changes in toxic action

were noted, indicating that both the carboxyl group and the hydrocarbon chain play a significant part in the toxic effect.

The introduction of a double bond in the hydrocarbon chain brings about certain important changes in the chemical and physical properties, and the determination of its effect seemed of some importance. Two unsaturated acids (undecenoic and oleic) were therefore included in the tests.

The methods employed for the determination of insecticidal values have been dealt with in previous papers (3, 4, 5), and a brief résumé is given in Part V of this series of papers (this vol., p. 217).

Experiments with *Aphis rumicis*.

In the case of the salts dealt with in this paper, which themselves cause a marked lowering of surface tension, we have made a departure from the usual technique and have used a 0·5 per cent. solution of saponin (in place of 1 per cent.) for dilution. The sodium salts of lauric, palmitic and stearic acids proved difficult to spray on account of the tendency of their solutions to form gels; this was got over in the case of sodium laurate by spraying at a slightly higher temperature, but no data were obtained with sodium palmitate and stearate.

The samples of the fatty acids available were not in all cases chemically pure. The acid content of each was therefore determined by titration and the necessary corrections made where the acid content was low enough to introduce a significant error. Except in the cases of lauric and undecenoic acids, the corrections required were quite small. The figures are given in Table I.

The salts were prepared by dissolving known amounts of each of the acids in the smallest quantity of alcohol necessary to bring to a clear solution and adding caustic soda or ammonia, until in the case of the sodium soaps the solution was slightly alkaline, and in that of the ammonium salts distinctly alkaline, to phenol phthalein. The solutions were then warmed, allowed to cool, and a little more alkali added if the colour did not return on cooling. No attempt was made to bring each solution to the same *p*H value.

After spraying, the insects are always placed within reach of fresh bean foliage and are examined on the two (or if necessary, three) following days. At each examination counts are made under four headings: "unaffected," "slightly affected," "moribund," and "dead." With the compounds discussed in this paper, the figures for the first day's count

are tabulated, except in the few cases when recoveries occurred after a further 24 hours. In the tables, except in the cases of unimportant compounds, the data for the four categories are given, but in the diagrams the sums of the figures in the last two categories only are plotted against the concentration.

It is true that the diagrams do not always show the finer changes in toxicity with change in concentration so well as the figures in the tables. They do however indicate satisfactorily the difference in toxicity existing between the compounds and, as the curves are not in any case suitable for purposes of statistical analysis, it has not been thought worth while to introduce a more detailed and laborious method of graphing the results.

In a previous paper (3) we have stated that the concentrations yielding a mortality of 50 per cent. are the most suitable for purposes of comparison as between the different compounds. It was noted, however, that with many of the fatty acids a slight decrease in concentration resulted in a profound quantitative change in toxic action and some of the curves decline so steeply that exploration about the region of the 50 per cent. point was impracticable; therefore, when comparisons were made between partition coefficients or the number of carbon atoms in the molecule and toxicity, we chose the lowest concentrations yielding 100 per cent. moribund and dead for the purpose, and have attempted, among the more highly toxic compounds, to determine this value with as great an accuracy as possible for purposes of correlation.

The Fatty Acids.

In Table I the results obtained for all the concentrations of all the acids tested are set out. In Diagram 1, in order to avoid too many inter-crossings, the values for the saturated, but not for the unsaturated acids are graphed.

An inspection of Table I and Diagram 1 shows that formic acid, while only slightly toxic, is more so than acetic. Formic acid cannot be regarded as a typical fatty acid, as in addition to the constitution of an organic acid it has an aldehydic structure which confers reducing properties and probably increases toxicity. Both Table and Diagram show that toxic properties steadily increase from acetic to decylic and undecylic acids. The difference in toxic values between propionic and butyric acids is only a small one, but there occurs a large gap between butyric and valerianic acids. Diagram 1 shows no significant difference in toxicity between decylic and undecylic acids, but dodecylic (lauric) and

Table I.

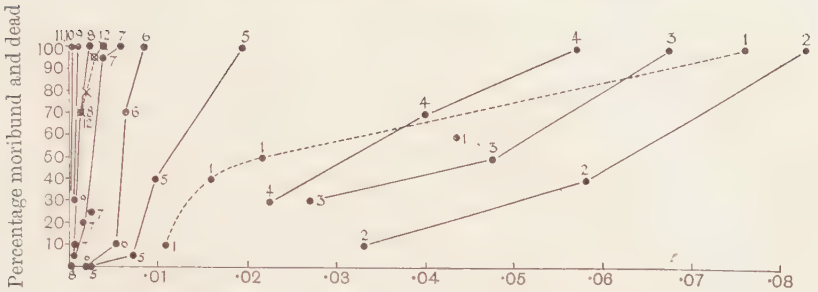
Toxicities of the Fatty Acids to A. rumicis (*1st Series*).

(N = Not affected. S = Slightly affected. M = Moribund. D = Dead.)

Name of acid	No. of tests	Concentration of acid: Gm. per 100 c.c.	Concentration of acid: Mole per 100 c.c.	N %	S %	M %	D %	M and D %
Formic.	1	5·0	0·109	—	—	—	100	100
(HCOOH.	1	3·5	0·076	—	—	—	100	100
No. of carbon atoms 1.	1	2·0	0·0435	20	20	—	60	60
Mol. wt. 46·02.	2	1·0	0·022	40	10	25	25	50
% acid content 97·8)	1	0·75	0·016	30	30	—	40	40
	1	0·5	0·0108	90	—	—	10	10
Acetic.	1	5·0	0·083	—	—	50	50	100
(CH_3COOH.	1	3·5	0·058	30	30	20	20	40
No. of carbon atoms 2.	1	2·0	0·033	50	40	10	—	10
Mol. wt. 60·04.	2	1·0	0·0166	75	15	5	5	10
% acid content 98·5)	1	0·5	0·0083	90	10	—	—	—
Propionic.	1	5·0	0·067	—	—	10	90	100
(C_2H_5COOH.	1	3·5	0·047	30	20	30	20	50
No. of carbon atoms 3.	1	2·0	0·027	60	10	30	—	30
Mol. wt. 74·06.	2	1·0	0·0135	90	5	5	—	5
% acid content 96·75)	1	0·5	0·0067	100	—	—	—	—
Butyric.	1	5·0	0·057	—	—	50	50	100
(C_3H_7COOH.	1	3·5	0·04	—	30	70	—	70
No. of carbon atoms 4.	1	2·0	0·022	20	50	20	10	30
Mol. wt. 88·08.	1	1·0	0·0113	50	20	20	10	30
% acid content 96·6)	1	0·75	0·0085	80	—	10	10	20
	1	0·25	0·0028	100	—	—	—	—
Valerianic (normal).	1	5·0	0·049	—	—	—	100	100
(C_4H_9COOH.	1	3·5	0·034	—	—	—	100	100
No. of carbon atoms 5.	1	2·0	0·0196	—	—	40	60	100
Mol. wt. 102·1.	1	1·0	0·01	20	40	40	—	40
% acid content not determined)	2	0·75	0·0073	80	15	—	5	5
	1	0·25	0·0025	100	—	—	—	—
Caproic (hexylic)	1	2·0	0·017	—	—	—	100	100
(isobutyl acetic).	1	1·0	0·0086	—	—	30	70	100
($C_5H_{11}COOH$.	2	0·75	0·0064	30	—	60	10	70
No. of carbon atoms 6.	1	0·5	0·0043	70	20	10	—	10
Mol. wt. 116·1.	2	0·25	0·0021	85	15	—	—	—
% acid content 98·75)								
Oenanthic (heptylic).	3	0·75	0·0057	—	—	—	100	100
($C_6H_{13}COOH$.	2	0·5	0·0038	5	—	45	50	95
No. of carbon atoms 7.	2	0·35	0·0027	40	35	15	10	25
Mol. wt. 130·1.	2	0·2	0·0015	45	35	20	—	20
% acid content 97·2)	2	0·1	0·00077	80	10	10	—	10
Caprylic (octylic).	2	0·75	0·0052	—	—	—	100	100
($C_7H_{15}COOH$.	2	0·5	0·0035	—	—	—	100	100
No. of carbon atoms 8.	2	0·35	0·0024	—	—	5	95	100
Mol. wt. 144·2.	2	0·22	0·0014	—	30	55	15	70
% acid content 99·15)	2	0·1	0·0007	95	—	—	5	5
	2	0·05	0·00035	95	5	—	—	—

Table I (*continued*)

Name of acid	No. of tests	Concentration of acid: Gm. per 100 c.c.	Concentration of acid: Mole per 100 c.c.	N %	S %	M %	D %	M and D %
Pelargonic (nonylic).	1	0·68	0·0043	—	—	—	100	100
($C_8H_{17}COOH$.	2	0·45	0·0028	—	—	15	85	100
No. of carbon atoms 9.	2	0·31	0·002	—	—	10	90	100
Mol. wt. 158·1.	2	0·18	0·0011	—	—	35	65	100
% acid content 91·55)	2	0·09	0·00057	35	35	20	10	30
	2	0·045	0·00028	80	20	—	—	—
Capric (decylic).	1	0·7	0·004	—	—	—	100	100
($C_9H_{19}COOH$.	2	0·46	0·0027	—	—	10	90	100
No. of carbon atoms 10.	2	0·32	0·0019	—	—	20	80	100
Mol. wt. 172·2.	2	0·186	0·00117	—	—	40	60	100
% acid content 92·85)	2	0·093	0·00054	—	—	65	35	100
	2	0·046	0·00027	25	40	35	—	35
	2	0·023	0·00013	65	30	5	—	5
Undecylic.	1	0·7	0·0037	—	—	—	100	100
($C_{10}H_{21}COOH$.	2	0·46	0·0024	—	—	—	100	100
No. of carbon atoms 11.	2	0·32	0·0017	—	—	—	100	100
Mol. wt. 186·3.	2	0·186	0·00098	—	—	40	60	100
% acid content 92·5)	2	0·093	0·00049	—	—	85	15	100
	2	0·046	0·00024	30	35	20	15	35
	2	0·023	0·00012	60	40	—	—	—
Lauric (dodecylic).	1	0·8	0·004	—	—	30	70	100
($C_{11}H_{23}COOH$.	4	0·6	0·003	2·5	2·5	72·5	22·5	95
No. of carbon atoms 12.	3	0·4	0·002	10·3	10·3	44·8	34·5	79
Mol. wt. 200·2.	3	0·28	0·0014	10	20	43·3	26·7	70
% acid content 80)	3	0·16	0·0008	13·3	36·7	30	20	50
	3	0·08	0·0004	66·7	20	6·6	6·6	13
Tridecylic.	5	4·5–0·67	0·021–0·0031					100
($C_{12}H_{25}COOH$.	1	0·45	0·0021	—	—	90	10	100
No. of carbon atoms 13.	1	0·36	0·0017	—	10	40	50	90
Mol. wt. 214·2.	1	0·315	0·0015	—	—	100	—	100
% acid content 90)	1	0·27	0·00126	—	10	70	20	90
	1	0·18	0·00084	40	30	30	—	30
	1	0·09	0·00042	80	—	10	10	20
Myristic (tetradecylic). ($C_{13}H_{27}COOH$. No. of carbon atoms 14. Mol. wt. 228. % acid content 92·1)	Not materially toxic at a concentration of 5·0 % = 0·02 mole per 100 c.c.							
Palmitic $C_{15}H_{31}COOH$ and Stearic $C_{17}H_{35}COOH$	Not materially toxic at a concentration of 5·0 % (0·0195 and 0·0175 mole per 100 c.c. respectively.							
Undecenoic.	5	0·645–0·3	0·0035–0·0016					100
($C_{10}H_{19}COOH$.	2	0·17	0·00092	—	—	90	10	100
No. of carbon atoms 11.	2	0·086	0·00046	15	—	60	25	85
Mol. wt. 184·27.	2	0·043	0·00023	5	55	30	10	40
% acid content 85·7)	1	0·021	0·00011	90	—	—	10	10
Oleic.	1	1·0	0·0035	—	—	—	100	100
($C_{17}H_{33}COOH$.	2	0·75	0·0026	—	5	10	85	95
No. of carbon atoms 18.	1	0·5	0·0018	30	—	30	40	70
Mol. wt. 282·36.	1	0·35	0·0012	50	30	10	10	20
% acid content 97·7)	1	0·2	0·0007	50	30	20	—	20



Concentration (corrected to acid content) in mole per 100 c.c.
The acids are specified by the number of carbon atoms in the molecule (see Table I).

Diagram 1. Toxicity of the fatty acids to *A. rumicis*.

tridecylic acids decline in toxicity to a small but probably significant extent. The acids higher than tridecylic are distinctly less toxic, myristic, palmitic and stearic showing no toxic action at any of the concentrations tested.

In order to fix more accurately the lowest concentration giving 100 per cent. mortality and to determine more exactly the change in toxicity with decreasing concentration, a fresh series of experiments with the more highly toxic acids was made, the concentrations used being brought as near together as conveniently possible. To avoid the possibility of effects due to secondary factors, benzene was not used in this series, the solid acids being dispersed in saponin solution in extremely fine condition. Lauric acid could not be used in this way as the finer particles aggregated at too great a rate. The results are given in Table II and Diagram 2 which is plotted on a larger scale than Diagram 1. In Diagram 2 the data for dodecylic (lauric) and tridecylic acids are taken from Table I.

The results of these experiments are in close agreement with those obtained previously and confirm the increase in toxicity with molecular weight from the 5 carbon-atom acid to the 11 carbon-atom acid, and demonstrate that on passing from decylic to undecylic acid there is a slight increase in toxicity.

The results agree well with the statement of Siegler and Popenoe (1) that "the toxicity of the fatty acids increases with the molecular weight, at least to a certain point not yet definitely determined. A practical amount of toxicity was reached with the sixth carbon-atom, the peak of toxicity apparently lying at or slightly above the C_{10} point."

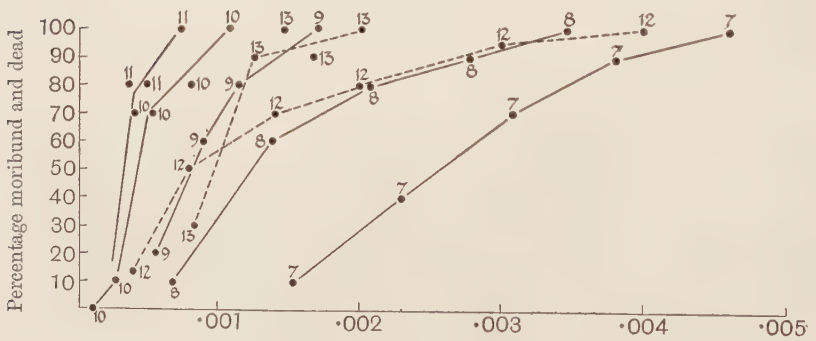
Table I shows the results obtained with the two unsaturated acids, undecenoic and oleic. The former is as toxic as the saturated acids,

Table II.

Toxicities of the Fatty Acids to A. rumicis (*2nd Series*).

(N = Not affected. S = Slightly affected. M = Moribund. D = Dead.)

Name of acid	Concentration of acid: Gm. per 100 c.c.	Concentration of acid: Mole per 100 c.c.	N %	S %	M %	D %	M and D %
Oenanthic (heptylic). Acid content 97·2 %	0·6	0·0046	—	—	—	100	100
	0·5	0·0038	—	10	20	70	90
	0·4	0·0031	10	20	30	40	70
	0·3	0·0023	50	10	40	—	40
	0·2	0·0015	90	—	—	10	10
Caprylic (octylic). Acid content 99·1 %	0·5	0·0034	—	—	—	100	100
	0·4	0·0028	—	10	10	80	90
	0·3	0·0021	—	20	40	40	80
	0·2	0·0014	20	20	60	—	60
	0·1	0·0069	80	10	—	10	10
Pelargonic (nonylic). Acid content 91·5 %	0·36	0·0023	—	—	10	90	100
	0·27	0·0017	—	—	30	70	100
	0·18	0·0011	—	20	70	10	80
	0·135	0·00085	—	40	50	10	60
	0·09	0·00057	20	60	20	—	20
	0·067	0·00042	50	30	10	—	10
Capric (decylic). Acid content 92·85 %	0·186	0·001	—	—	30	70	100
	0·14	0·0008	—	20	50	30	80
	0·093	0·00054	—	30	70	—	70
	0·07	0·0004	10	20	50	20	70
	0·046	0·00027	70	20	—	10	10
	0·023	0·00013	90	10	—	—	—
Undecylic. Acid content 92·5 %	0·186	0·00098	—	—	10	90	100
	0·14	0·00074	—	—	30	70	100
	0·093	0·00049	—	20	60	20	80
	0·07	0·00037	—	20	80	—	80
	0·046	0·00024	70	30	—	—	—
	0·023	0·00012	80	—	20	—	20



Concentration in mole per 100 c.c. (corrected to true acid content).

The acids are specified by the number of carbon atoms in their molecules (*e.g.* 7 = heptylic).

Diagram 2. Toxicities of the fatty acids (heptylic to dodecylic) to *A. rumicis*.

undecylic and decylic, the slight differences being scarcely significant. Oleic acid is less toxic than undecenoic acid.

The Sodium and Ammonium Salts of the Fatty Acids.

Solutions of the sodium and ammonium salts of the fatty acids were prepared in the manner already described (p. 333) and dilutions made up in the usual way, except that the strength of the saponin solution was reduced to 0·5 per cent. It seemed advisable for compounds like the soaps, in which surface activity is so pronounced, to reduce the strength of the wetting reagent. In two sets of experiments carried out with sodium oleate, with and without the addition of saponin, the results obtained were in very close agreement; in each case a concentration of 0·75 per cent. (reckoned as acid) resulted in a mortality of 100 per cent. and 0·5 per cent. gave 90 per cent. of deaths.

The data for the *sodium salts* are not given completely except in the case of sodium oleate. Conversion of the acid to the sodium salt greatly reduces toxicity as a rule; thus, stating the results in terms of the acid, pelargonic acid (nonylic) was completely toxic at a concentration of 0·2 per cent. whereas the corresponding sodium soap was scarcely toxic at 1 per cent.; capric acid (decylic) and undecylic acid were toxic at 0·1 per cent., the sodium salts barely toxic at 1·0 per cent. With lauric acid (dodecylic) and myristic acid (tetradecylic), the effect of converting to the sodium salts was not so pronounced, sodium laurate being scarcely less toxic, and sodium myristate apparently more toxic, than the corresponding acid; but in both these cases, particularly with the myristate, there was a tendency for the insects to be glued down by the material, with consequent injury. Further, if the figures for sodium oleate are compared with those for sodium undecenoate, it will be seen that the effect of saponification in reducing toxicity is less marked among the highest members of the series; thus, whereas undecenoic acid is completely toxic at 0·2 per cent., the sodium soap is not toxic at all at a concentration (calculated as acid) of 0·5 per cent. and scarcely toxic at 1·0 per cent. Oleic acid and sodium oleate on the other hand show hardly a significant difference and, if anything, the soap is slightly more toxic. It was unfortunately impossible to compare the sodium palmitates and stearates with the corresponding acids, as their solutions are gels at ordinary temperatures even at fairly low dilutions and were not suitable for spraying.

The results obtained with the *ammonium salts* of the acids, ranging from caproic to stearic, are expressed in Table III, which also includes

sodium oleate; and in Diagram 3 the results for the ammonium salts from caproic to lauric acid are plotted.

Table III.

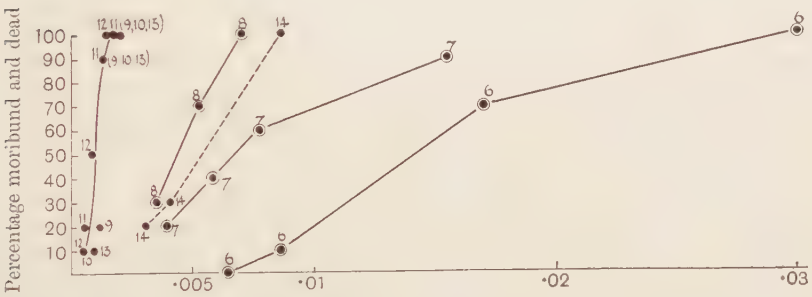
Toxicities of the Ammonium Salts of the Fatty Acids to A. rumicis.

(N = Not affected. S = Slightly affected. M = Moribund. D = Dead.)

Name	Concentration of acid: Gm. per 100 c.c.	Concentration of acid: Mole per 100 c.c.	N %	S %	M %	D %	M and D %
Ammonium caproate (hexylate)	3·5	0·03	—	—	30	70	100
	2·0	0·017	30	—	10	60	70
	1·0	0·0086	90	—	—	10	10
	0·75	0·0064	90	10	—	—	—
Ammonium oenanthate (heptylate)	2·0	0·0154	10	—	—	90	90
	1·0	0·0077	20	20	20	40	60
	0·75	0·0057	40	20	30	10	40
	0·5	0·0038	80	—	10	10	20
	0·35	0·0027	70	10	—	20	20
	0·2	0·0015	100	—	—	—	—
Ammonium caprylate (octylate)	1·0	0·007	—	—	—	100	100
	0·75	0·0052	20	10	20	50	70
	0·5	0·0035	60	10	10	20	30
	0·35	0·0024	50	20	—	30	30
	0·2	0·0014	100	—	—	—	—
Ammonium pelargonate (nonylate)	0·9	0·0054	—	—	—	100	100
	0·68	0·0043	—	—	—	100	100
	0·45	0·0028	—	—	10	90	100
	0·31	0·002	—	—	40	60	100
	0·225	0·0014	—	10	20	70	90
	0·18	0·0011	50	30	10	10	20
	0·09	0·00057	80	20	—	—	—
Ammonium caprate (decylate)	0·46	0·0027	—	—	10	90	100
	0·35	0·002	—	—	30	70	100
	0·23	0·0013	—	10	50	40	90
	0·093	0·00054	80	10	10	—	10
Ammonium undecylate	0·93–0·46	0·0049–0·0024					100
	0·32	0·0017	—	—	10	90	100
	0·23	0·0012	—	10	30	60	90
	0·093	0·00049	30	50	10	10	20
Ammonium laurate (dodecylate)	2·0–0·4	0·01–0·002					100
	0·28	0·0014	—	—	40	60	100
	0·16	0·0008	40	10	50	—	50
	0·08	0·0004	60	30	—	10	10
	0·04	0·0002	80	20	—	—	—
Ammonium tridecylate	0·45	0·0021	—	—	40	60	100
	0·31	0·0014	—	20	30	50	80
	0·18	0·00084	80	10	—	10	10
	0·09	0·00042	90	10	—	—	—
Ammonium myristate (tetradecylate)	1·84	0·008	—	—	30	70	100
	0·92	0·004	50	20	10	20	30
	0·69	0·003	60	20	—	20	20
	0·46	0·002	60	20	10	10	20
	0·32	0·0014	90	10	—	—	—

Table III (*continued*).

Name	Concentration of acid: Gm. per 100 c.c.	Concentration of acid: Mole per 100 c.c.	N %	S %	M %	D %	M and D %
Ammonium palmitate	Not toxic, at a concentration of 2·0 % of the acid = 0·0078 mole per 100 c.c.						
Ammonium stearate	Not toxic at a concentration of 2·0 % of the acid = 0·007 mole per 100 c.c.						
Ammonium undecenoate	0·63	0·005	—	—	—	100	100
	0·43	0·0023	—	10	10	80	90
	0·21	0·0011	—	10	20	70	90
	0·086	0·00046	60	30	—	10	10
Ammonium oleate	2·5–0·75	0·0088–0·0026					100
	0·5	0·0018	—	—	—	100	100
	0·35	0·0012	20	—	—	80	80
	0·2	0·0007	20	10	—	70	70
	0·1	0·00035	100	—	—	—	—
Sodium oleate	2·5 and 1·0	0·0088–0·0035					100
	0·75	0·0026	—	—	10	90	100
	0·5	0·0018	5	5	5	85	90
	0·35	0·0012	20	10	—	70	70
	0·2	0·0007	40	10	—	50	50
	0·1	0·00035	40	30	—	30	30
Ammonia control	Not toxic, at 9·0 %						
0·5 and 1·0 % saponin solutions	Not toxic						
Alcohol and 0·5 % saponin solutions	Not toxic						



The compounds are specified by the number of carbon atoms in the acid (*e.g.* the line drawn through 6 indicates the results for ammonium caproate (hexylate)).

Diagram 3. Toxicities of the ammonium salts of the fatty acids to *A. rumicis*.

The neutralisation of the acid radical with ammonia decreases toxicity in certain of the acids. The reduction of toxicity due to neutralisation with ammonia is again less in the case of the higher fatty acids from nonylic upwards, ammonium tridecylate being of the same order of

toxicity as tridecylic acid and ammonium myristate markedly more toxic than myristic acid. It is probable that the toxicity of the ammonium salts is at least partly determined by the liberation by hydrolysis of the free fatty acids in a very finely divided state.

The ammonium salt of myristic acid (tetradecylic) is, however, less toxic than the ammonium salts of the acids of lower molecular weight (nonylic to tridecylic), while ammonium palmitate and stearate, at the highest concentration at which they could be conveniently tested, show no toxicity. As the same relationships are found amongst the acids themselves, it would appear that in this series molecular complexity, or some property depending on it, limits toxic action.

The Methyl Esters of the Fatty Acids.

The results obtained for the methyl esters of the fatty acids are given in Table IV and Diagram 4.

With the exception of methyl caprate, the toxicities are uniformly distinctly lower than those of the acids or the ammonium salts. Our tests may have exaggerated the toxicity of methyl caprate. Our sample was a very small one and the data in this case are probably not so good as in the rest of the tests. Nevertheless our sample of methyl caprate proved somewhat less toxic than the acid, mole for mole. The whole of the results demonstrate that the effect of methylation is to lower toxicity, and that there is an increase in toxicity as one ascends the series from methyl caproate (hexylate) to methyl caprate (decylate); methyl undecylate was not available, but methyl undecenoate shows a slight increase in toxicity over the caprate but is slightly less toxic than the corresponding acid, mole for mole. Methyl laurate (dodecylate) is less toxic than its immediate predecessors in the series and methyl stearate is not toxic at any concentration up to 5·0 per cent. (= 0·017 mole per 100 c.c.). The other noteworthy feature of the series is the comparatively high toxicity of methyl formate; this compound proved completely toxic at 0·083 mole per 100 c.c., whereas the acetate, propionate, butyrate and valerianate are not toxic at very high concentrations.

Certain of the benzyl esters were tested. The results suggest that where the acid is of small molecular weight the benzyl group has a preponderating effect upon toxicity, whereas with the acids of higher molecular weight the effect of this radical diminishes. As certain important benzyl esters were not available, the data with regard to this series is hardly sufficient to warrant further discussion at this stage.

Table IV.

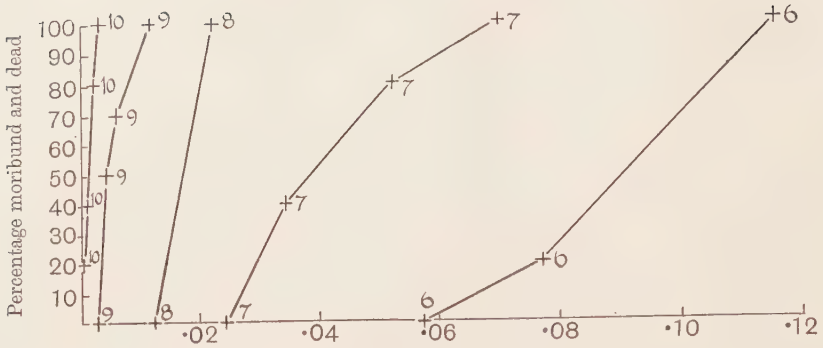
Toxicities of the Methyl Esters of the Fatty Acids to A. rumicis.

(N = Not affected. S = Slightly affected. M = Moribund. D = Dead.)

Name	Concentration of ester: Gm. per 100 c.c.	Concentration of ester: Mole per 100 c.c.*	N %	S %	M %	D %	M and D %
Methyl formate.	5·0	0·083	—	—	—	100	100
($H.COOCH_3$.	3·5	0·058	40	20	10	30	40
Mol. wt. = 60)	2·0	0·033	50	10	10	30	40
Methyl acetate (M.W. 74)	Not toxic, at a concentration of 20 %						
,, propionate (,, 88)	,,		,,		,,		
,, butyrate (,, 102)	,,		,,		,,		
,, valerianate (,, 116)	,,		,,		15 %		
Methyl caproate (hexylate)	15	0·115	—	—	50	50	100
($C_5H_{11}COOCH_3$.	10	0·077	—	80	20	—	20
Mol. wt. = 130·1)							
Methyl oenanthate (heptylate)	15	0·104	—	—	—	100	100
($C_6H_{13}COOCH_3$.	10	0·069	—	—	20	80	100
Mol. wt. = 144·1)	7·5	0·052	—	20	20	60	80
	5·0	0·034	10	50	—	40	40
	3·5	0·024	30	70	—	—	—
Methyl caprylate (octylate)	10–5·0	0·063–0·031					100
($C_7H_{15}COOCH_3$.	3·5	0·022	—	—	50	50	100
Mol. wt. = 158·1)	2·0	0·012	60	10	20	10	30
Methyl pelargonate (nonylate)	2·0	0·011	—	—	10	90	100
($C_8H_{17}COOCH_3$.	1·0	0·006	—	30	60	10	70
Mol. wt. = 172·2)	0·75	0·0043	30	20	50	—	50
	0·5	0·003	50	50	—	—	—
Methyl caprate (decylate)	2·0 and 1·0	0·0107 and 0·0053					100
($C_9H_{19}COOCH_3$.							
Mol. wt. = 186·2)	0·75	0·004	—	—	70	30	100
	0·5	0·0027	—	—	90	10	100
	0·35	0·0019	10	10	80	—	80
	0·2	0·001	30	30	40	—	40
	0·1	0·0005	70	10	20	—	20
	0·05	0·00027	80	20	—	—	—
Methyl laurate (dodecylate)	5·0 and 3·5	0·023 and 0·016					100
($C_{11}H_{23}COOCH_3$.							
Mol. wt. = 214·25)	2·0	0·0093	—	—	—	100	100
	1·0	0·0046	10	20	10	60	70
	0·75	0·0035	20	10	—	70	70
	0·5	0·0023	—	—	—	—	30?†
	0·25	0·0016	70	10	20	—	20
Methyl stearate ($C_{17}H_{35}COOCH_3$)	Not toxic at concentration of 5·0 % = 0·0176 mole per 100 c.c.						
Methyl undecenoate	2·0–0·75	0·01–0·004					100
($C_{10}H_{19}COOCH_3$.	0·5	0·0025	—	—	40	60	100
Mol. wt. = 198·2)	0·35	0·0017	—	10	90	—	90
	0·2	0·001	—	20	80	—	80
	0·1	0·0005	40	60	—	—	—
Methyl oleate	5·0	0·017	—	—	—	100	100
($C_{17}H_{33}COOCH_3$.	3·5	0·012	—	—	30	70	100
Mol. wt. = 296·3)	2·0	0·0067	20	—	50	30	80
	1·0	0·0033	40	20	30	10	40
	0·75	0·0025	50	40	—	10	10

* The concentrations in moles are the same whether expressed as acid or ester.

† Experiment spoilt owing to escape of insects.

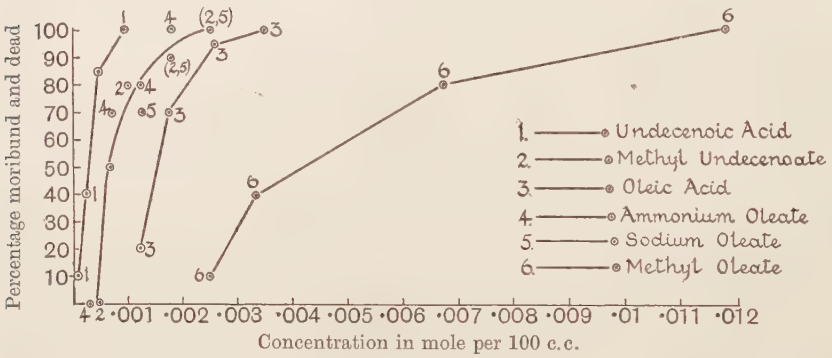


Concentration in mole per 100 c.c.

The methyl esters are specified by the number of carbon atoms in the acid (*e.g.* 6 = methyl caproate (hexylate)).

Diagram 4. Toxicities of the methyl esters of the fatty acids to *A. rumicis*.

Diagram 5 sets out the results obtained with the unsaturated acids (undecenoic and oleic) and their ammonium salts and methyl esters. Ammonium undecenoate is omitted in order not to obscure the diagram; it is slightly less toxic than the acid. The toxicities of methyl unde-



Concentration in mole per 100 c.c.

Ammonium undecenoate is not included; it was somewhat less toxic than undecenoic acid.

Diagram 5. Toxicities of undecenoic and oleic acids, their ammonium and sodium soaps and methyl esters, to *A. rumicis*.

cenoate, ammonium and sodium oleate at different concentrations (points 2, 4, 5) can be substantially represented by one curve. The diagram shows methyl undecenoate (2) less toxic than its acid, and methyl oleate less toxic than oleic acid. This acid is slightly less toxic than either its sodium or ammonium salts.

Diagram 6 shows the relationship between the insecticidal values of the acids, their ammonium salts and methyl esters and the molecular

weight of the respective acid which is represented by the number of carbon atoms present. The comparisons are made at concentrations giving 100 per cent. moribund and dead[1].

The diagram clearly indicates the increases in toxicity with molecular complexity, formic acid and its derivatives being exceptional throughout the three series; and in addition shows that the ammonium salts containing 6, 7 and 8 carbon atoms are less toxic than the corresponding

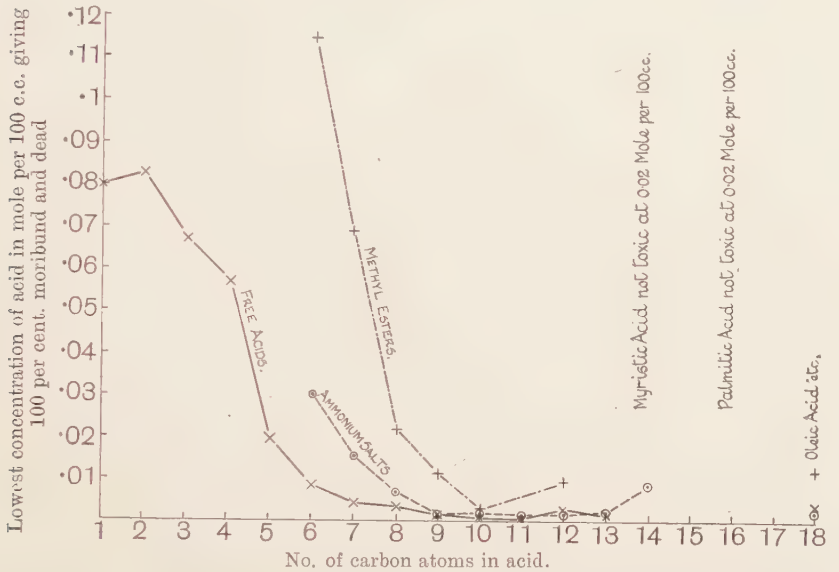


Diagram 6. Showing relationship between toxicity to *A. rumicis* of the fatty acids, their ammonium salts and methyl esters, and the number of carbon atoms in the molecule of each acid.

acids, but that from this point onwards to the 13 carbon-atom acid the toxicity of the ammonium salt approximates to that of its acid, and at 14 (myristic) and 18 (oleic) the ammonium salt is more toxic. The methyl esters are uniformly less toxic than the corresponding acids. Speaking generally, these facts indicate that both the hydrocarbon chain and the carboxyl group play a significant part in determining the intensity of the toxic action of this group.

[1] In the case of ammonium oenanthate (heptylate), the percentage giving a death rate of 90 per cent. has been taken, the value for exactly 100 per cent. not having been obtained.

Experiments with Eggs of *Selenia tetralunaria*.

A series of experiments was carried out to ascertain whether the fatty acids showed any toxicity to eggs of the moth *Selenia tetralunaria* Hüfn. The general method of procedure has been described in earlier papers. The acids were tested at concentrations ranging from 5·0 per cent. to 0·25 per cent., and the liquids for spraying were prepared by dissolving the acid in a small amount of benzene and emulsifying with the required amount of a 1 per cent. solution of saponin in water. Control sprayings were made with benzene (5–50 per cent.) and saponin solutions. The results are summarised in Table V.

Table V.

Toxicities of the Fatty Acids to the Eggs of Selenia tetralunaria.

Name of acid	Concentration in gm. per 100 c.c.	No. of tests	No. of eggs sprayed	No. of eggs not hatching	% eggs not hatching	% viable eggs killed (allowing for control)
Controls	—	14	1676	68	4·04	—
Caproic	2·0–0·25	4	480	18	3·75	0
Oenanthic	2·0	1	169	96	56·8	54·8
„	1·0–0·25	3	289	14	4·5	0
Caprylic	2·0	1	163	108	66·2	64·65
„	1·0	1	102	15	14·7	11·1
„	0·5	1	100	10	10·0	6·2
„	0·25	1	99	3	3·03	0
Pelargonic	2·0	1	127	102	80·3	79·3
„	1·0–0·25	3	325	14	4·3	0
Capric	2·0–0·25	4	446	16	3·6	0
Undecylic	2·0	1	167	72	43·1	40·6
„	1·0–0·25	3	314	13	4·1	0
Lauric	5·0–0·5	4	369	14	3·8	0
Myristic	5·0–1·0	3	329	15	4·2	0
Undecenoic	2·0	1	110	60	54·6	52·6
„	1·0–0·5	2	210	12	5·7	1·75
„	0·25	1	156	5	3·2	0
Oleic	5·0	1	114	91	79·8	78·8
„	2·5	1	75	44	58·7	56·85
„	1·0	1	145	9	6·2	2·25
„	0·5	1	114	7	6·12	2·2
Stearic	5·0	1	86	11	12·8	9·1

The control figures for the percentage of eggs which did not hatch, irrespective of treatment (4·04 per cent.), is the mean of the results obtained from six lots of unsprayed eggs, four lots sprayed with 1 per cent. saponin solution and four lots sprayed with different concentrations

of benzene in 1 per cent. saponin solution—1676 eggs in all. The maximum range of variation in these controls was between 7·05 and 1·33 per cent. of eggs not hatching. This control figure is allowed for[1] in calculating the percentage of viable eggs killed, given in the last column of Table V. The figures show that none of the acids had any marked toxicity to these eggs at concentrations below 2 per cent. It is interesting however to compare the results obtained with the different acids at this concentration (2 per cent.) as set out in Diagram 7.

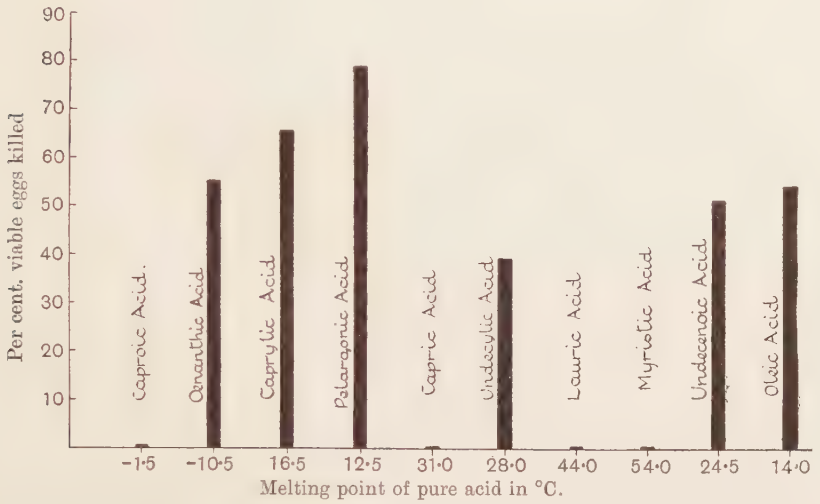


Diagram 7. Relative toxicities of fatty acids at a concentration of 2 per cent. to eggs of *Selenia tetralunaria*.

As the series is ascended from caproic to pelargonic acid, there is a rise in toxicity with increase in molecular weight; with the higher acids this no longer holds and only undecylic, undecenoic and oleic acids show any considerable toxicity. In attempting to account for these irregularities among the higher members of the series, it is perhaps suggestive that it is those acids with low melting points, *i.e.* those which are liquid at summer temperatures, which show toxicity. Penetration may take place more readily in the liquid state. Our figures would indicate undecylic acid to be rather exceptional in this respect. Our sample, however, under summer conditions, readily remained in a super-cooled state, and had a melting point slightly below that of the pure acid. It thus falls

[1] The average percentage which did not hatch in the controls (4·04 per cent.) is deducted from the percentage not hatched in each test and multiplied by $\frac{100}{100 - 4{\cdot}04}$.

satisfactorily into line with the above suggestion. Observations on the stage of development reached by the majority of the unhatched eggs in each of these experiments give some additional evidence for this point of view. It was noted that in the case of undecenoic and oleic acids used at a concentration of 2 per cent., nearly all the unhatched eggs had failed to develop much beyond the point reached when they were sprayed, whereas with undecylic acid at the same concentration the majority of unhatched eggs contained almost fully developed larvae. It is therefore probable that undecenoic and oleic acids have greater penetrating powers than undecylic acid and that the difference between the effects of these acids is even greater than is indicated on the diagram. For a similar reason, the difference between the toxicities of oenanthic and pelargonic acids is probably understated in the diagram and table.

Molecular Weight in Relation to Insecticidal Action.

The correlation between the weight of the molecule and the toxicity of the fatty acid is not complete. The higher toxicity of formic acid as compared with acetic shows the importance of the constitution of the molecule; oleic acid is also more toxic than stearic although of almost the same molecular weight, and in many cases the methyl esters are less toxic than the acids of corresponding molecular weight (see Diagram 6). Moreover in passing up the series a point is reached where toxicity declines, thus myristic, palmitic and stearic acids show little or no toxic action. Either, therefore, there is some molecular weight value at which inhibition of toxicity takes place, or more probably certain physical properties come into play which act as limiting factors.

Some Physical Properties considered in Relation to Insecticidal Action.

In the following sections an attempt is made to see whether any marked correlation exists between toxicity to insects and certain physical properties of the fatty acids. Except in regard to compounds in the vapour phase, there is little published work on the subject of possible relationships between physical properties and insecticidal action. On the other hand, there is a considerable amount of information available as to the bearing of physical properties on narcotic action, rate of penetration through cell walls and other physiological phenomena. In certain respects these problems have some analogy or parallelism with the problem of insecticidal action and, without attempting to review

the extensive literature, we have made reference to certain facts and theories advanced in the study of these problems, which seem to have a bearing on our results.

Physical State.

There is some evidence that compounds show a difference in the intensity of their toxic action to insects according to the physical state in which they are applied.

The data presented in a previous section on the toxicity of these acids to the eggs of *S. tetralunaria* suggest that the solid acids are less toxic than the liquid acids and that there is some correlation between toxicity and the melting point, *e.g.* capric acid (solid) is less toxic than pelargonic acid (a liquid) (see p. 346). It is not, however, clear that this consideration applies to the toxicity of these acids to aphids, for although such solid acids as myristic, palmitic and stearic show only a slight toxicity when compared with the liquid acids pelargonic, caprylic and oleic, yet capric acid (decylic), a solid, is more toxic than pelargonic (nonylic), which is a liquid at ordinary temperatures. It may be that the toxic effect of solid suspensoids is determined by the size of particle. The compounds of high melting point are somewhat difficult to obtain in a condition of very fine suspension, and there is a tendency for the particles to aggregate. As finely divided particles are more rapidly soluble than coarse ones, and as the lower melting point acids are more soluble in organic media than those of a higher melting point, the factor directly involved may be concerned with solubility in the cell membranes rather than with physical state. Consideration is given to solubility relationships in a later section.

Volatility.

It has been shown by Moore(7) and by Tattersfield and Roberts(6) that there is a rough correlation between the boiling points, volatilities and vapour pressures of compounds and the toxicity of their vapours to insects. Within certain limits, the lower the volatility or vapour pressure, the greater the insecticidal action. Among similarly constituted compounds this is no doubt generally true, for it is reasonable to suppose that the more readily a vapour is adsorbed, the greater its insecticidal action, and "the adsorption on a carbon surface increases with the boiling point of the adsorbed gas[1]."

In the case of insecticides applied as sprays, the application of a

[1] Harkins, *Colloid Symposium Monograph*, II, 166.

similar conception is complicated by solubility and surface tension relationships. A highly volatile compound would presumably be less effective than a less volatile one, as it would evaporate more readily from the surfaces of the insect and have less time to act before being dissipated. This may account for the toxicities of methyl acetate, propionate and butyrate being apparently abnormally low. The relationship does not, however, always hold, since myristic and palmitic acids are less toxic than the more volatile acids, capric, pelargonic and caprylic; and further it is affected by considerations of chemical constitution, for example, the highly volatile methyl formate is more toxic than methyl butyrate.

Dissociation Constants.

The rate at which a compound penetrates would appear to be a determining factor in insecticidal action. The literature concerning the rate of penetration of the acids has grown to considerable dimensions, and although there are few instances where the experimental conditions correspond with ours, a brief résumé of some of the conclusions arrived at is of interest.

Adrian Brown(8) showed that the covering of the seeds of *Hordeum vulgare* shows a selective permeability to acids; it is not so readily penetrated by mineral as by organic acids. This property does not seem to be associated with the degree of dissociation, as relatively strong acids such as formic and chloracetic penetrate readily.

Loeb(9) found that mineral acids less readily induced membrane formation and were less rapidly toxic to the unfertilised eggs of sea-urchins than were the fatty acids. The greater the number of carbon atoms in the latter, the shorter the time necessary for membrane formation, a result intelligible on the assumption that those acids which diffuse most rapidly into the eggs call forth this response most readily. The conclusion drawn was that the acids diffuse into the cell only as undissociated molecules, particularly as certain of the sodium salts of the fatty acids gave negative results.

Harvey(10) found no correlation between the rate of penetration of the acids into the pigmented gonodial filaments of *Stechopus ananus* Jaeger, and the degree of ionisation; whereas Crozier(11), from his experiments on the blue pigmented membranes of *Chronoderis zebra* Heilprim, considered ionisation to be a primary factor in determining cell permeability for acids, and that the degree of dissociation, if sufficiently great, practically controls the situation, but if sufficiently low,

as in the fatty acid series (formic acid excepted), some factor allied to capillary activity is dominant.

The dissociation constants for certain of the fatty acids are included in Table VI; and it is clear from this table that among the fatty acids dissociation constants cannot be correlated with insecticidal action. Formic acid, a fairly strong acid, is more toxic than acetic on the one hand, but less toxic than most of the higher fatty acids, and although the dissociation constants from acetic to nonylic (pelargonic) are all of the same order, there is nevertheless an increasing degree of toxicity.

Partition Coefficients.

Meyer(12) in 1899 and Overton(13) in 1901 advanced the view that narcotic action was connected with the partition coefficients, *i.e.* the ratio of the solubility of the narcotic in oil or lipoid to that in water. This theory has been subjected to a considerable amount of investigation and criticism. Bayliss(14) has pointed out that, although the theory shows how a narcotic obtains access to the cell, "it does nothing more in explanation of its action than to suggest that it is in some way exerted on the boundary membrane," the lipoid constitution of which seems to have been widely accepted. It is generally considered that the theory is too specific to give an altogether satisfactory account of narcotic action. It is, in the main, an attempt to explain rate of penetration into cells. Nevertheless, this theory is of importance from our point of view, since readiness of penetration is probably a determining factor in insecticidal action. The results obtained by Phillipson(15) showed that the rates of passage of certain acids through collodion membranes impregnated with a complete etherial extract of muscle were in the following order: butyric > lactic > acetic > formic, but that the membrane was impermeable to mineral acids. Harvey (*loc. cit.*) considered that the penetration of the acids depended on several factors, *e.g.* lipoid solubility, capillary activity, and an affinity for certain proteins of the cell surface. The results obtained by Loeb and by Crozier (*loc. cit.*) also indicated the importance of the Overton-Meyer theory. We therefore determined the partition coefficients as between olive oil and water of the fatty acids used in our experiments.

Determination of the partition coefficients. Olive oil of B.P. quality containing about 1 per cent. of free acid was used. A quantity of acid approximately equivalent to $M/100$ was dissolved in the oil at 25–26° C., the exact concentration being determined by titration, allowing for the

free acid in the olive oil. In the case of myristic acid it was necessary to use $M/200$ on account of its low solubility. The oil solutions were then shaken up vigorously several times in stoppered cylinders with the same volume of water at 25° C. and allowed to stand in a constant temperature room at 25–26° C. for several weeks. The aqueous layer

Table VI.

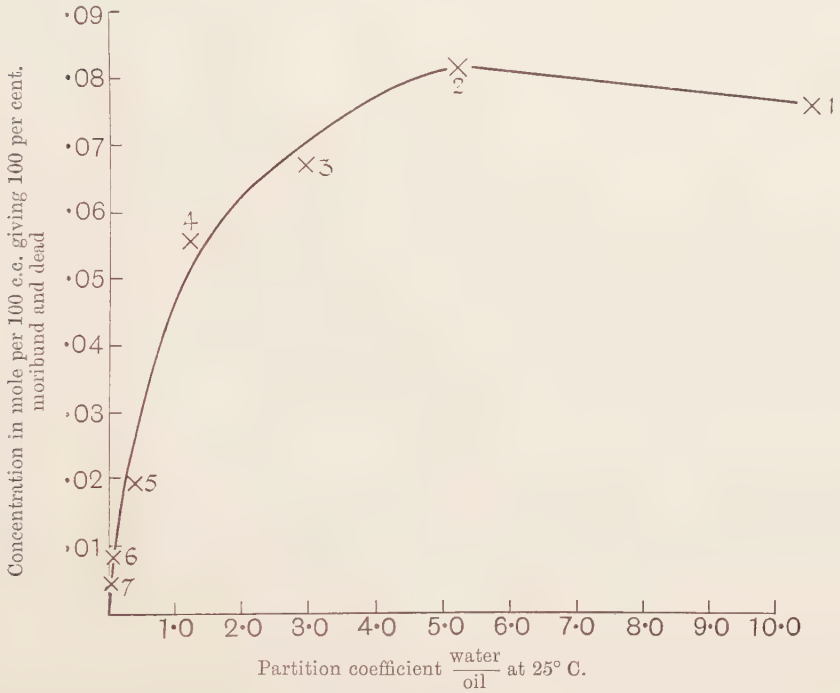
Fatty Acids. Partition Coefficients for Water and Olive Oil, Dissociation Constants and Effect on Surface Tension of Water.

No. of carbon atoms	Name	Mol. wt.	A Oil layer Acid content before extraction gm./100 c.c.	A Oil layer Acid content after extraction gm./100 c.c.	B Aqueous layer Acid content after extraction gm./100 c.c.	Partition coefficients Oil/water A ÷ B	Partition coefficients Water/oil B ÷ A	Dissociation constants
1	Formic	46	0·398	0·0345	0·363	0·095	10·5	$2·14 \times 10^{-4}$
2	Acetic	60	0·591	0·096	0·495	0·19	5·15	(25° C.) $1·8 \times 10^{-5}$
3	Propionic	74	0·705	0·18	0·525	0·34	2·9	„ $1·3 \times 10^{-5}$
4	Butyric	88	0·883	0·405	0·482	0·83	1·2	„ $1·5 \times 10^{-5}$
5	Valerianic	102	1·018	0·738	0·28	2·64	0·38	„ $1·6 \times 10^{-5}$
6	Caproic	116	1·19	1·06	0·13	8·15	0·12	„ $1·4 \times 10^{-5}$
7	Oenanthic	130	1·27	1·234	0·036	34·27	0·029	„ $1·4 \times 10^{-5}$
8	Caprylic	144	1·419	1·404	0·015	91·7	0·011	„ $1·4 \times 10^{-5}$
9	Pelargonic	158	1·85	1·845	<0·0046	>401·2	<0·0025	„ $1·1 \times 10^{-5}$
10	Capric	172·2	1·677	1·675	<0·0016	>1009	<0·001	—
11	Undecylic	186·2	1·71	1·697	<0·0129	Aq. solution turbid		—
12	Lauric	200·2	1·78	1·78	Trace	Very large	Very small	—
13	Tridecylic	214·2	1·92	1·918	<0·002	Aq. solution turbid		—
14	Myristic	228·3	0·975	0·975	Trace	Very large	Very small	—
15	Isocetic	242·3	Not available					
16	Palmitic	256·3	1·7	1·7	—	Very large	Very small	—
17	Not available							
18	Stearic	284·4	—	—	—	—	—	—
Unsaturated acids:								
11	Undecenoic	184·3	1·585	1·572	<0·0128	Aq. solution turbid		—
18	Oleic	282·3	2·63	2·63	Trace	Very large	Very small	—

The surface tensions at 25° C. of the saturated solutions in water of the following acids were in dynes/cm:—Capric 32·64, undecylic 47·1, lauric 70·1, palmitic 71·2, oleic 71·3, water being taken as 71·8. (Traube's stalagmometer used.)

was carefully syphoned off in such a manner as not to draw over more than a trace of the oil layer, and allowed to stand for some time in corked tubes till clear, when a known quantity of the clear liquid was pipetted off and titrated with either $N/50$ or $N/100$ sodium hydroxide solution. In the case of undecylic, undecenoic and tridecylic acids, the aqueous layer maintained a persistent turbidity and the results could

not be taken. From the values obtained from the oil layers (before aqueous extraction) and those obtained from the final aqueous layers the partition coefficients could be determined. The determination of the amounts of the higher fatty acids dissolved in water can only be regarded as approximate[1].



The acids are specified by the number of carbon atoms in the molecule (×1 = formic acid).

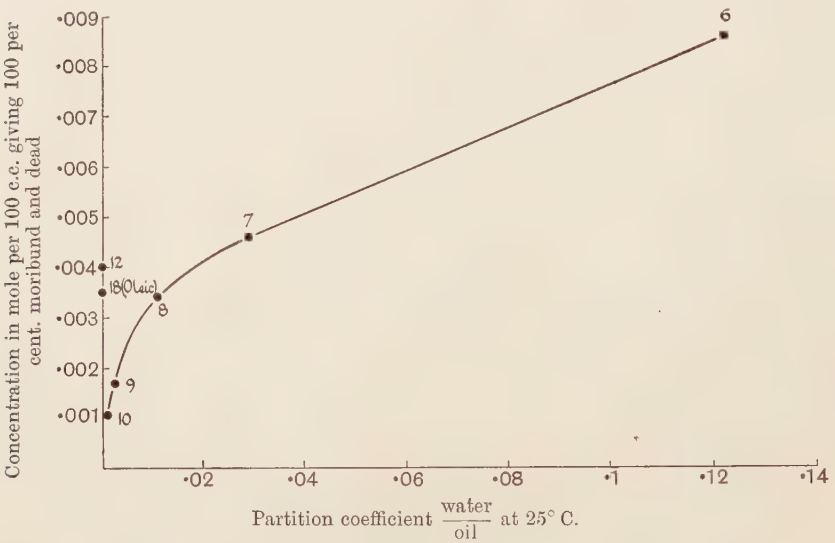
Diagram 8. Showing relationship between toxicities of the fatty acids to *A. rumicis* and their partition coefficients $\frac{\text{water}}{\text{oil}}$. (See also Diagram 9.)

The results are expressed in Table VI both as oil/water and water/oil solubility-ratios. In Diagrams 8 and 9 the water/oil solubility ratio is plotted against the lowest molar concentrations of the respective acids yielding 100 per cent. moribund and dead. This gives figures suitable for graphical representation.

The results are expressed in two diagrams of different scales, as the range of partition coefficients is so wide as to render obscure the results obtained for the higher acids if all are expressed on the same diagram.

[1] A surface tension method could not be applied under our conditions.

Diagram 8 gives a conspectus of the results for the acids formic (1 carbon atom) to oenanthic (7 carbon atoms). With the exception of formic acid, there is a steady rise in toxicity with a decrease of the partition coefficient (water/oil solubility ratio). Diagram 9 shows a similar close correlation from oenanthic to capric acid (10 carbon atoms). (Undecylic is omitted as the aqueous solution had a persistent turbidity rendering the determination unsatisfactory.) In the case of lauric acid[1] (12 carbon atoms) and of oleic acid (18 carbon atoms), there is a break



The acids are specified by the number of carbon atoms in the molecule (6 = hexylic, 18 = oleic).

Diagram 9. Showing relationship between toxicities of the fatty acids to *A. rumicis* and their partition coefficient $\frac{\text{water}}{\text{oil}}$.

in the correlation between toxicity and partition coefficient. They are both less toxic than would be anticipated on the assumption that the correlation held.

The coefficients, however, merely express the degree of separation of the acid between oil and water and are no measure of the absolute solubilities in either of the two media. As there is, in general, a decline in the solubilities of the solid acids in organic solvents with increase in molecular weight, this should probably be taken into account in considering any relationship between toxicity and the partition coefficient.

[1] The results of the spraying tests with lauric acid were less concordant than with the other acids; the average figures have been taken.

The differences in solubility in oil might explain the low toxicities of myristic, palmitic and stearic acids when compared with, say, decylic, and of stearic when compared with oleic, but they do not account for the low toxicity of oleic acid as compared with undecenoic, decylic or nonylic, oleic acid being miscible in all proportions with oil.

The Overton-Meyer theory does not distinguish clearly between the uses of oil and lipoid in determining the partition coefficient, and the question arises whether a liquid fat like olive oil can be legitimately substituted for a solid or semi-solid lipoid of the type of lecithin, which possesses hygroscopic qualities and complex solubility relationships[1]. A hydrophilic colloid of this type is capable of adsorbing certain water-soluble substances. The water-solubility relationships of the narcotic or toxic substances are brought into prominence by this consideration. The fatty acids have a group (carboxyl) that is definitely polar to water, a fact which has led to the modern theories of Langmuir and Harkins in regard to the orientation of these acids in the surface of their aqueous solutions. In addition they possess, in their hydrocarbon chain, a group definitely polar to products of the type of the organic solvents. Both of these properties are possibly of importance in determining passage through and adsorption by certain types of membranes and hence the toxicity of these compounds. The experiments of Adrian Brown (*loc. cit.*) suggested to him that the different rates of diffusion of the acids through the coverings of the barleycorn were due to some unrecognised peculiarity in which the molecules of the two classes of solutes were combined with the molecule of solvent water. It is perhaps suggestive that in our experiments saponification of the lower fatty acids, which materially increases their solubility in water, decreases toxicity; this effect declines as the length of the hydrocarbon chain increases until in the case of oleic, the ammonium and sodium soaps are, if anything, more toxic than the acid.

It may therefore be concluded that despite the fairly close correlation between the toxicities of many of the acids and the partition coefficients, the application to insecticidal action of the Overton-Meyer theory of narcotic action does not afford a complete explanation of our results. It cannot be applied without consideration of chemical constitution[2] and other physical properties. Among the fatty acids the correlation is,

[1] Most of the work since Overton and Meyer indicates that the cell surface is more complex than is represented on the lipoid theory.

[2] *E.g.* methylation of the carboxyl group which presumably decreases the values of the partition coefficients water/oil results in a general decline in toxic properties.

however, suggestively close and there is obviously need for further work on the relationships existing between solubility, penetration and toxic action.

Surface Tension.

It has been realised for some considerable time that surface tension relationships are of significance, and indeed are probably one of the determining factors in the problems of permeability, penetration and narcotic action. In a long series of papers since 1904, Traube (16) has stressed the importance of this physical property with respect to osmosis and penetration. He has applied his "cohesion pressure[1]" (*haftdrück*) theory to problems of narcosis, disinfection and the physiological action of drugs and poisons. The Overton-Meyer theory of narcosis is criticised on the grounds that lipoid-free cells can be subjected to the action of narcotics (17), an objection not applicable to his theory, which is largely based upon the view that compounds with the property of lowering surface tension will cause a change in the physical state of the cell contents, with a consequent retardation of certain metabolic processes such as oxidation. Czapek (18) has shown that surface tension effects are of considerable importance in determining the penetration of the fatty alcohols and other organic chemicals into living cells. Working with several of the higher plants, he showed that penetration took place when the aqueous solutions of the alcohols attained a critical value of about 0·68–0·69 of the surface tension of water.

It was realised by both Harvey and Crozier (*loc. cit.*) that capillary activity might be one of the factors intimately bearing on the problem of the penetration of acids. The surface tensions of the aqueous solutions of fatty acids from formic to decylic (with the exception of octylic) have been thoroughly worked out by Harkins, Clark and King, and very complete data have been published by King (19). King shows that from formic to decylic the relative lowering of the surface tension of water increases with the molecular weight of the acid; thus a solution of 18·6 moles of formic acid per litre causes a reduction at 20° C. from 72·8 to 41·2 dynes/cm., whereas a reduction to approximately the same figure is produced by decylic acid at a concentration of only ·0002 mole per litre. There is on the whole a similarity between the adsorption curves of King and our curves relating toxicity with concentration as shown in Diagrams 1 and 2. The data given by King (*loc. cit.*) do not go beyond decylic acid, and his experiments show that the true surface

[1] The more a substance lowers the surface tension of a solvent, the smaller its cohesion pressure when dissolved in it.

tension value of the solution of this acid is not reached in the drop-weight method until a period of 30 minutes has been allowed for the solution to come into equilibrium with its surface. Frumkin(20) estimated that solutions of lauric acid require several hours. A few preliminary determinations of the surface tension of the aqueous solution of the higher acids have been made by us, but much further work is required before deductions can be drawn in regard to the relationship between surface activity and the insecticidal action of these compounds. Using a Traube stalagmometer, with which only a short time can be allowed for surface equilibrium to be established, we observed that from lauric acid upwards, the surface tensions of the saturated solutions approximated to that of pure water. It may be suggestive that toxicity among the acids begins to decline with lauric acid, but this decline could not be correlated in any simple way with the surface tension of the aqueous solutions of the respective acids. The lowered capillary activity under these conditions is due to the solubility of these acids being very low and the molecules sparsely distributed in the solution. This leads to the question already raised (p. 355) as to whether the toxicity of these acids is not in some way bound up with water-solubility relationships.

Summary.

1. The toxicities to *Aphis rumicis* L. of the fatty acids from formic to stearic and of the sodium and ammonium salts and methyl esters, applied as spray fluids, have been quantitatively determined. Two unsaturated acids, undecenoic and oleic, are included.

2. There is a rise in toxicity of the acids with increase of molecular weight as the series is ascended from acetic to undecylic acid. Formic acid is exceptional. Beyond undecylic acid, there is a fall in toxicity, and acids higher in the series than tridecylic show only slight toxic action.

3. The sodium salts of the fatty acids are in most cases much less toxic than the corresponding acids, though the difference is less marked with the higher acids. Oleic acid and sodium oleate are of the same order of toxicity.

4. The ammonium salts are also generally less toxic than the corresponding acids, but the differences are much less than in the case of the sodium salts. With some of the higher acids, *e.g.* myristic and oleic, neutralisation with ammonia increases toxicity. The relatively high toxicity of the ammonium salts may be due, at least partly, to liberation, by hydrolysis, of free fatty acid in a very finely divided state.

5. Methylation of the fatty acids reduces toxicity; all the methyl esters are less toxic than the acids or ammonium salts.

6. Both the ammonium salts and the methyl esters show, like the acids themselves, increase of toxicity with increase of molecular weight up to a certain point. The formates are exceptional.

7. The fatty acids do not show marked toxicity to the eggs of *Selenia tetralunaria* Hüfn. at concentrations below 2 per cent.

8. Possible relationships between certain physical properties (physical state, volatility, dissociation constants, partition coefficients and surface tension) of the fatty acids and their insecticidal action are discussed.

9. Determination of partition coefficients as between olive oil and water and comparison of the figures with the relative toxicities show a steady rise in toxicity with a decrease in the partition coefficients (water/oil) from acetic to capric acid. Formic acid is again exceptional. With lauric and oleic acids there is a break in the correlation. The bearing of the solubility relationships of the acids on these results is considered.

LITERATURE CITED.

(1) Siegler, E. H. and Popenoe, C. H. (1924). *Journ. Agric. Res.* xxix, 259.
(2) —— —— (1925). *Journ. Econ. Ent.* xviii, 292.
(3) Tattersfield, F. and Morris, H. M. (1924). *Bull. Ent. Res.* xiv, 223.
(4) Tattersfield, F., Gimingham, C. T. and Morris, H. M. (1925). *Ann. App. Biol.* xii, 61.
(5) —— —— —— (1925). *Ann. App. Biol.* xii, 218.
(6) Tattersfield, F. and Roberts, A. W. R. (1920). *Journ. Agric. Sci.* x, 199.
(7) Moore, W. (1917). *Journ. Agric. Res.* x, 365.
(8) Brown, A. J. (1907). *Ann. Bot.* xxi, 79.
—— (1909). *Proc. Roy. Soc.* B, lxxxi, 82.
(9) Loeb, J. (1913). *Artificial Parthenogenesis.* (Univ. of Chicago.) Chap. xiv, 133.
(10) Harvey, E. N. *Internat. Zeit. phys.-chem. Biol.* i, 462. (*Science* (1914), xxxix, 947.)
(11) Crozier, W. J. (1916). *Journ. Biol. Chem.* xxiv, 255.
(12) Meyer, H. H. (1899). *Arch. Exp. Path.* xlii, 109.
(13) Overton, E. (1901). *Studien über die Narkose.* Jena.
(14) Bayliss, W. M. (1924). *Principles of General Physiology*, p. 140.
(15) Phillipson, M. and Hannevart, G. (1920). *Compt. Rend. Soc. de Biol.* lxxxiii, 1570.
(16) Traube, I. (1904). *Pflüg. Archiv Physiol.* cv, 541.
Traube, I. and Somogyi, R. (1921). *Biochem. Zeit.* cxx, 90.
(17) Traube, I. (1913). *Pflüg. Archiv Physiol.* cliii, 276.
(18) Czapek, F. (1910). *Ber. Bot. Ges.* xxviii, 480.
(19) King, H. H. (1922). *Techn. Bull.* 9, *Kansas Agric. Exp. Sta.*
(20) Frumkin, A. (1925). *Z. physik. Chem.* cxvi, 466.

(*Received November 30th*, 1926.)

INVESTIGATIONS ON THE CONTROL OF WIREWORMS

By HERBERT W. MILES, M.Sc. (*Bristol*), N.D.A. and F. R. PETHERBRIDGE, M.A. (*Cantab.*).

(With Plates XXIV and XXV and 2 Text-figures.)

CONTENTS.

		PAGE
I.	The Wireworm Problem	359
	Historical	359
	Observations on wireworm attacks	362
	Feeding habits of wireworms in relation to weeds	364
	Seasonal movements of wireworms	365
II.	Baiting as a Means of Assembling Wireworms	368
	Field experiments—1925	368
	Field experiments—1926	373
	Experiments in glasshouses—1925–6	374
	Experiments on baiting amongst growing crops	376
	Conditions for successful baiting	378
III.	Baiting and Calcium Cyanide for Wireworm Control	379
	Preliminary tests	380
	Field Experiments in Lincolnshire—1925	380
	Experiments in glasshouses—1926	382
	Experiments amongst growing crops—1926	383
	Cost of treatment	383
IV.	Conclusions and Recommendations	385
V.	Summary	386
	Bibliography	387

I. The Wireworm Problem.

Historical.

Wireworms cause considerable annual losses amongst agricultural and horticultural crops, and though these losses are especially severe for the first three or four years after land has been broken up from grass or ley, more or less injury occurs year by year when the wireworm content has reached its normal level. In general farming areas such crops as turnips, swedes, potatoes, barley and oats are particularly susceptible

to attack, while in horticultural areas tomatoes and chrysanthemums frequently suffer severely.

During the Great War the demand for increased food production led to the breaking up of grassland and concentrated the attention of farmers and others on injurious soil insects. Applications for advice to the Board of Agriculture and the Advisory Centres indicated that the wireworm was one of the most troublesome and common of soil pests and the wireworm problem was studied by several research workers in an effort to obtain fuller information and if possible discover suitable methods of control.

The susceptibility of various kinds of garden crops to injury by wireworm was studied by A. H. Lees at the Long Ashton Research Station (8). Lees studied the vegetable crops grown on land within two years of breaking up and grouped them into classes according to the degree of susceptibility to wireworm injury. Thus plants like onions, leeks, celery and lettuce which were attacked at the fleshy collar, and killed, dwarfed or caused to go to seed prematurely were classed as "very susceptible." Plants like runner beans, dwarf beans and peas were "rather susceptible," being dwarfed but not usually killed. The cabbage tribe and tomatoes were "slightly susceptible," but the former ran less risk as the plants were fairly hard about the collar at the time of planting out. In a fourth group, wireworm injury did not endanger the life of the plants: thus with potatoes the injury was confined to the tubers (Pl. XXIV, fig. 1), considerably decreasing their market value but the collar of the plant escaped; carrots also would belong to this group. The practical application of these findings was that crops on new land could be selected with due regard to the probability of wireworm attack, and highly susceptible crops avoided.

A. Roebuck (19), Harper Adams Agricultural College, studied the wireworm content of grass fields and newly broken up fields, keeping the latter under observation each year until they passed into normal cultivation. He found by calculation that certain grass fields near Newport, Salop, contained from 215,000 to 510,000 wireworms per acre, the majority located within the surface two inches of soil (1). Roebuck also studied cultural treatment for the control of wireworms, noting that cultivation and systems of cropping exercised considerable influence on the pest.

G. H. Ford (4), Manchester University, made a study of the larval and pupal stages of *Agriotes obscurus* L., the larvae of which are the most common wireworm in Cheshire, North Staffordshire and South Lancashire.

Probably by far the most important investigation was that initiated at the Rothamsted Experimental Station in 1916 by A. W. Rymer Roberts (16), who studied in great detail the life history and structure of wireworms of the genus *Agriotes* and made some observations on those of *Athous haemorrhoidalis* F., rearing the former species from egg to adult which took from August 1916 to the middle of 1921, and determining that under English conditions the length of life in the larval stage may be of six years' duration.

Much was added to our knowledge of wireworms by the research carried out from 1915 to 1920 but no definite means of controlling the pest was developed, though some attention was paid to control by cultural means by Harper Gray (12) and others, and local trials with soil insecticides, chiefly of a proprietary nature, were carried out. Tattersfield and Roberts (21), however, studied the toxicity of organic compounds to wireworms and found that compounds with irritating vapours have usually high toxic values, *e.g.* allyl isothiocyanate, chloropicrin benzyl chloride, while chemically inert compounds boiling above 170° C. are generally uncertain in their poisonous effect on wireworms after an exposure of 1000 minutes at 15° C.

Post-war conditions, far from stimulating work on the control of soil pests, have adversely affected it and the general tendency has been for farmers to lay more land down to grass. In areas where intensive culture is followed and where industrial areas have provided stimulus for market growing, land is, however, constantly being broken up from grass or ley. Here the wireworm problem is still serious and the demand for effective control measures urgent; for potatoes, cruciferous crops and beans in intensive areas; strawberries in fruit-growing areas; peonies, pyrethrums, asters, chrysanthemums and certain other flowers in market growing areas; and tomatoes and other crops under glass, are all liable to severe injury by the depredations of this pest.

In the autumn of 1925 investigations on the control of wireworms (10), (11) were commenced, and these have now reached a stage when definite recommendations can be made. The following is a complete account of the work.

The writers acknowledge their indebtedness for suggestions and advice to Mr J. C. F. Fryer, M.A., Director, Ministry of Agriculture Pathological Laboratory, and for assistance to Mr W. G. Kent, N.D.H., Horticultural Superintendent, Isle of Ely. Thanks are also due to Mr Frank Waite of Boston, Mr Ivor Mard of Hatfield and Mr Yates

Christie of Worthing, for their kindness in placing land or glasshouses at our disposal for the investigation.

(2) *Observations on wireworm attacks.*

Since wireworms are most troublesome when grassland is broken up the general practice is to grow special crops for two or three years before adopting the normal rotations followed in the district. Thus oats, potatoes, and mustard are often selected during the first few years. From the writers' observations oats appear to be less susceptible to wireworm attack than either wheat or barley; they tiller freely and, even when attack is serious, make sufficient tillers to carry a crop and yield fairly satisfactory returns. Potatoes do well on new land and after a crop of potatoes there is generally a marked diminution in the wireworm content of the soil, presumably owing to large numbers being removed with the crop. There is some risk of the seed tubers being attacked but when land is newly broken up there is usually sufficient undecayed turf in the soil to provide food for the wireworms for some time. In Lincolnshire it was frequently noticed that early potatoes on newly broken up land escaped attack by wireworms while late crops under similar conditions were often severely infested, probably owing to exhaustion of other food. Mustard usually does well on new land and suffers very little from the attacks of soil insects, hence it is commonly taken as a first crop in Lincolnshire and followed by potatoes the next year.

In fruit-growing areas where land is broken up for strawberry culture it is customary to take one crop, usually potatoes, before planting out strawberries. For the first year or two after planting, however, some proportion, often a high one, is lost through wireworm depredations. The wireworms tunnel into the young crowns and cause death to the growing point, or gnaw at the young roots so weakening the plants that many finally perish.

Where flower culture is followed losses often occur especially among cuttings and young plants. In chrysanthemum-growing districts—West Sussex, Hampshire, Cambridge and Norfolk—the usual practice is to plant rooted cuttings out in the field. The wireworms tunnel up the stems of these causing considerable loss. In the autumn the surviving plants are taken into the glasshouses, with the result that wireworms may be introduced amongst the roots, and their attack on subsequent crops like tomatoes often results in the loss of from a quarter to a third of the plants. Asters and stocks pricked out as seedlings also suffer from wireworm attacks; and pyrethrums, which otherwise do

exceptionally well on new land, have often to be replanted. In May 1926 examination of 18 pyrethrum plants on a flower farm in Cambridgeshire revealed a total of 18 wireworms present; 10 of the plants were free from the pest but 4 of the remaining plants had 3 wireworms at the roots of each, 2 had 2 wireworms each and 2 had 1 each. Carnations too, frequently suffer from wireworm depredations and here again the characteristic tunnelling up the stem is apparent.

Tomatoes in glasshouses often suffer severely from attack by wireworms. In a case which came to the writers' notice records showed that out of 2196 plants 580 were destroyed by wireworms, and several growers state that they have lost more than half their plants owing to wireworm attacks. In a block of glasshouses near Worthing where tomatoes and chrysanthemums were grown alternately, 10 representative soil samples, 6 in. square and 18 in. deep, taken at the end of the fourth tomato season, yielded 9 wireworms: by calculation, a wireworm content of 156,816 per acre. Careful examination of the attacked plants show that the wireworms most commonly enter the stems just below the surface-feeding adventitious roots and tunnel upwards from 5 to 8 in. (Pl. XXIV, fig. 2), causing the plant to become dwarfed, greyish in colour and wilted, finally inducing death. In cases of high infestation 5 or 6 wireworms per stem may be found, the attack becoming apparent within a day or two after setting out the plants.

An interesting case of wireworm attack on vines was observed in Middlesex. Young vines were planted in October 1925 in a properly prepared vine border to which a quantity of new turf had been added. The vines did not make good headway and eventually the trouble was traced to wireworms which were destroying the roots as fast as they were being produced.

During the winter of 1924–5 the writers examined numbers of swede seed plants which were suffering from various bacterial rots and in many cases the plants had been first attacked by wireworms, often several wireworms occurring in characteristic tunnels in the plants. Mangold plants set out for seed were found attacked in a similar manner, especially during mild periods in the winter months.

The nature of wireworm attack on ordinary farm crops is so well known as to need no special description. The wide range of plants in both general agriculture and commercial horticulture which may suffer from the depredations of this pest bears out the truth of Curtis's statement(3) that "in the wireworm we have an example of a larva which

may be termed omnivorous as far as regards the productions of the field and garden."

The wireworms occurring most commonly during the writers' investigations in the County of Holland, Lincolnshire, were the larvae of *Agriotes obscurus* L. Odd specimens of the larvae of *Athous haemorrhoidalis* F. occurred occasionally at the roots of strawberries and crops grown in bays between fruit trees. The larvae of *Dolopius marginatus* L. also occurred in Lincolnshire and were often taken during 1924–5 feeding at the roots of cabbages and cauliflowers. The wireworms met with in glasshouses were the larvae of *Agriotes obscurus* L. and *A. sputator* L. These two species were found in numbers in two localities in Hertfordshire and in West Sussex.

(3) *Feeding habits of wireworms in relation to weeds.*

In order to obtain information on the natural food preferences of wireworms, a survey of the weeds on an arable field in Lincolnshire known to be infested with wireworms was made in the autumn of 1925. Wherever possible 100 specimens of each species of weed were examined but in many cases it was impossible to find that number. Each plant with its roots and the surrounding soil was carefully lifted and shaken and the number of wireworms found was recorded. The figures are given in the following table:

Table I.

Species	No. examined	Wireworms
Agropyrum repens	100	56
Taraxacum officinale	100	20
Senecio vulgaris	100	15
Plantago lanceolata	100	20
Poa annua	100	7
Stellaria media	100	4
Bromus sp.	100	3
Chenopodium album	100	1
Urtica urens	54	0
Sonchus spp.	34	3
Cardamine hirsuta	24	2
Achillea millefolium	14	21
Miscellaneous grass seedlings	11	2
Dactylus glomerata	10	1
Urtica dioica	7	0
Rumex acetosa	5	0
Polygonum persicaria	4	0
Veronica chamaedrys	3	0
Potentilla anserina	1	0

The observations indicated that the relation between the wireworms and the plant was influenced largely by the character of the underground portion of the plant. The moist and fibrous nature of the underground portions of yarrow and couch grass proved particularly attractive; the tap roots of dandelions were less so; and dry, fibrous roots like those of chickweed, goutweed and nettle seemed to be avoided. The specimens of groundsel were large and had well-developed roots and those of *Plantago* also had good compact root systems. In addition to the weeds, volunteer potatoes and small cabbage plants were also examined with interesting results; 100 small potato plants yielded 114 wireworms and 100 cabbage plants 31 wireworms.

(4) *Seasonal movements of wireworms.*

Wireworms may be found within a few inches of the surface of the soil at almost any season of the year, but there are certain well-marked periods when they are particularly prevalent. In two plots at Rothamsted, Herts, examined periodically from February 1920 to January 1921, Morris(13) found wireworms each month, and of the total wireworms taken 32·2 and 32·6 per cent. were located in the soil from 5 to 7 in. deep. In Aberystwyth area in 1920–21 M. Thompson(23), sampling soil from June to the following May, found wireworms within the top 3 in. of soil each month except August, the highest number for a sample occurring in April. Hammond(5) arrived indirectly at the seasonal prevalence of wireworms by examination of the crops of 10 starlings each month for a year, the maximum numbers of wireworms occurring in the crops in April, and the minimum during the summer months. In like manner Theobald and McGowan(22) found that wireworms occurred in greatest numbers in March and October to December.

Observations on the winter movements of wireworms in the soil were taken by the writers from October 1925 to March 1926. Soil samples 6 in. square were examined in three layers: the surface 3 in., the section 3–6 in. deep and the section 6–9 in. deep (vide Table II).

Expressed graphically (Fig. 1) the figures show high peaks in October and March for the top 3 in. of soil, with a depression between them corresponding to a high peak in the 6–9 in. which reached its apex in December and fell off from January to March. In other words there appeared to be a steady migration of wireworms down deeper into the soil in October, followed by fluctuations, probably in response to local conditions of temperature and moisture, and a steady upward movement from January to March. From observations taken in the

Table II.

Date	No. of samples containing wireworms	Wireworms present in		
		Surface 3 in.	3–6 in.	6–9 in.
Oct. 4 and 5	17	19	6	9
„ 15	5	6	4	0
„ 19	8	6	7	1
„ 29	10	6	7	1
Nov. 6	10	5	8	3
„ 14	10	0	7	4
„ 20	10	0	5	9
„ 24	10	2	4	5
„ 27	3	0	1	2
Dec. 4	10	0	4	11
„ 19	10	2	6	7
„ 28	10	1	10	6
Jan. 2	10	0	5	8
„ 9	10	1	11	8
„ 15	10	0	5	8
„ 23	10	1	4	7
„ 30	10	1	6	7
Feb. 6	10	1	8	4
„ 13	10	1	6	4
„ 20	10	3	7	4
„ 27	10	3	5	4
Mar. 6	10	4	2	4
„ 13	10	7	7	3
„ 20	10	11	6	4
„ 27	10	7	5	2

field there seems to be another migration of the wireworms downwards in summer, again probably in response to temperature and moisture conditions. Thus crops which have been seriously checked by wireworm attack often progress satisfactorily in June and July, and during the period from June to August it is often difficult to find wireworms except at some depth in the soil. In a series of soil samples taken in a glasshouse at Worthing in September the majority of wireworms were located at from 12 to 15 in. deep and some occurred 15 to 18 in. deep. The temperature at 15 in. deep was 58° F. while at the surface the soil temperature was 60° F. and the top few inches of soil were very dry, conditions likely to occur in the open at midsummer. With the advent of autumn these soil conditions change and an upward migration of wireworms is again apparent.

Seasonal movements of wireworms have an important bearing on control measures, for operations carried out in mid-summer or mid-winter

would be less likely to yield successful results than operations carried out in spring or autumn when the maximum number of wireworms occur in the surface soil. Moreover in mid-summer the land is usually under crops and cannot be disturbed while in mid-winter the wet and frozen

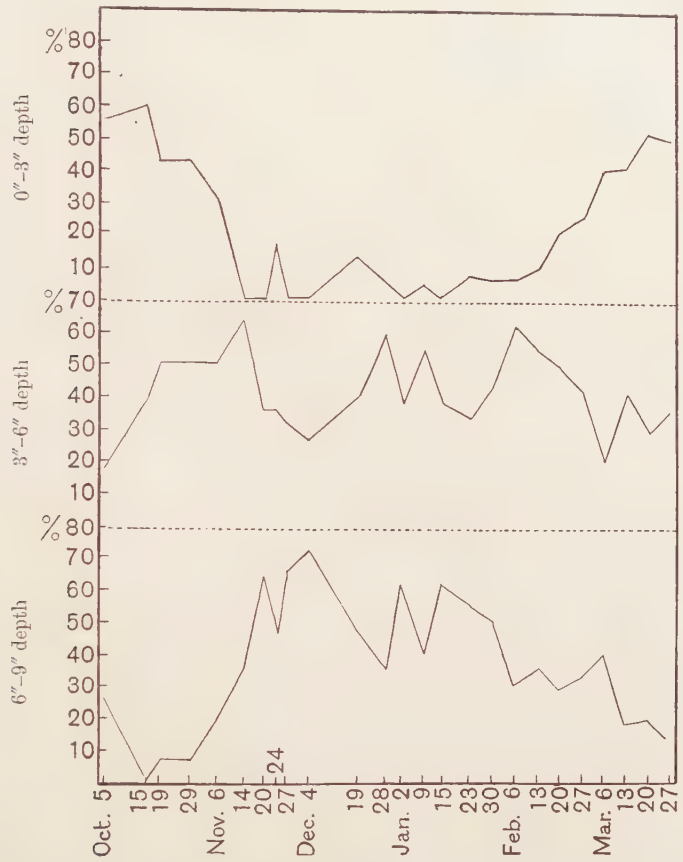


Fig. 1. Graph showing seasonal movements of wireworms from data obtained in Lincolnshire, October 1925–March 1926.

condition of the soil would render cultivation impossible; but in spring and autumn, prior to cropping and after the removal of the crops, routine cultivation can be made use of in connection with the control of wireworms.

II. Baiting as a Means of Assembling Wireworms.

Ormerod (14) and Miall (9) discuss the use of rape dust and rape cake for attracting wireworms and the former mentions that when a dressing of these substances is applied to a crop the wireworms leave the crop to feed upon the rape. Potatoes are mentioned by Curtis (3) as a suitable bait for attracting wireworms; and Wardle and Buckle (24) give a brief account of field work in California where potato baits, planted in lines down the field and periodically examined, resulted in the trapping of as many as 4000 wireworms per acre. Since then (1913) little was done on the use of baits until the discovery of substances toxic to wireworms, but too expensive for widespread use under general farming conditions, drew the attention of American entomologists to the possibility of using bait as a means of assembling wireworms into well defined areas when treatment could be applied at a greatly reduced cost. This was referred to in 1924 by Campbell (2), who suggested that split beans and rice bran might be used for this purpose, and in October 1925 Spuler (20) published an account of work done in Washington on the use of such baits as bran, potatoes, carrots, peas, beans and maize. He arrived at the conclusion that germinating seeds attract more wireworms than any other bait; moreover, seeds are easily planted and the bait rows can be readily traced.

(1) *Field Experiments* 1925.

In October 1925 the writers commenced investigations at Boston, Lincolnshire, to test the efficiency of baiting as a means of assembling wireworms and to compare the merits of various substances for use as bait. The experiments were carried out in a field lent for the purpose by F. Waite, Esq., of Boston. The field had been broken up early in the spring of 1925 and a crop of early potatoes grown. Later cabbages had been drilled but these were a failure, so that save for a few cabbage plants and odd weeds of the species listed on p. 364 the land was fallow. The soil was typical Lincolnshire silt with a considerable amount of more or less undecayed turf, and here and there were pockets of soil of a heavier nature. A portion of the field about 4 acres in extent was selected for the trials and the soil sampled for wireworms, 83 samples each 6 in. square and 9 in. deep yielding 81 wireworms, or approximately 170,000 per acre, the majority located within the top 3 in. of soil.

The land was first cleared of weeds and lightly worked with a hoe; then from October 5th to 12th 21 rows, each 10 yd. long and 5 ft. apart,

were set out with baits at a depth varying from 2–4 in. The baits, selected according to cheapness and availability, included chopped potatoes, oats, bran, wheat, peas and beans. They were examined after a period of 14 days, the whole row, 6 in. wide and 6 in. deep, being taken out and sifted, and the assembled wireworms removed and their numbers recorded. The following table gives a list of the baits used, indicates the order of the bait rows and shows the results obtained.

Table III.

Row No.	Bait	Depth of planting in.	Wireworms attracted
1	Potatoes	4	41
2	Oats	2–3	89
3	Wheat	2–3	88
4	Potatoes	3	24
5	Beans	2–3	51
6	Peas	2–3	33
7	Potatoes	2	15
8	Grass	4	34
9	Oats	2–3	32
10	Wheat	2–3	46
11	Grass	3	55
12	Beans	2–3	41
13	Peas	2–3	42
14	Grass	2	37
15	Bran	4	64
16	Oats	2–3	38
17	Wheat	2–3	47
18	Bran	3	52
19	Beans	2–3	34
20	Peas	2–3	62
21	Bran	2	47

The results obtained from this experiment indicated that wireworms could be readily attracted to baits. In the total area baited 972 wireworms were assembled, by calculation about 13,000 per acre, in 14 days from the setting of the bait. A comparison of the total number of wireworms attracted to each bait is interesting; wheat attracted 181 wireworms, bran 163, oats 159, peas 137, beans 126, grass 126, and potatoes 80.

The influence of depth of baiting on the assembling of wireworms was shown by the preceding experiment. A comparison of similar baits set at varying depths is given in Table IV.

Table IV.

Bait	Depth in.	No. of wireworms	Total
Bran	2	47	
Grass	2	37	99
Potatoes	2	15	
Bran	3	52	
Grass	3	55	131
Potatoes	3	24	
Bran	4	64	
Grass	4	34	139
Potatoes	4	41	

This indicated that bait planted at a depth of 3–4 in. was more efficient than that planted 2 in. deep.

The next experiment was designed to compare the rates at which wireworms were attracted to various baits. With the exception of grass, which was difficult to handle, baits similar to those used in the previous experiment were set 2–3 in. deep in rows 6 ft. apart and 20 yd. long, the series being planted in duplicate. Each day a sample 1 yd. long, 6 in. wide and 4 in. deep was sifted out from each row, the first examination occurring 24 hours after the setting of the baits. Table V and Fig. 2 give the results obtained over a period of 24 days.

This experiment indicated that bran and wheat attract wireworms most speedily, and that oats, peas, beans and potatoes increase in attractiveness from 6 to 12 days after setting. The attractiveness of bran for the wireworms decreased after about 12 days; peas and beans showed a fairly steady attraction during the last 18 days, and potatoes showed rather poor attraction throughout the entire period. The outstanding results were obtained from wheat and oats, which showed considerable attraction during the last 18 days of the period, assembling large numbers of wireworms during the final week of the experiment. The data obtained from this experiment suggested that the time available for baiting would have some bearing on the bait chosen. Where only a short time could be spared bran or wheat or a mixture of both might be set, or if a longer period were available oats might be selected as bait.

In order to secure information as to the distance over which baits would prove attractive, two experiments were carried out; the first with field beans set in rows at varying distances up to 6 ft. apart, and the second with wheat drilled in rows at varying distances up to 10 ft. apart. The beans were sown on October 7th and examined 14 days later, and the wheat was drilled on October 25th and examined on November 14th. The rows were sifted out in the usual way and the number of wireworms

Table V.

Attractiveness of Wireworm Baits.

Bait	Date examined October: 13	14	15	16	17	19	20	21	22	23	24	Total 1st 12 days	October: 26	27	28	29	30	31	November: 2	3	4	5	Total 2nd 12 days	Total for period
	Wireworms attracted												Wireworms attracted											
Bran	4	3	2	6	6	6	10	9	9	11	66	2	5	4	1	2	0	1	0	0	1	16	82
Oats	6	1	1	2	2	3	2	6	6	2	31	10	1	15	11	5	22	14	5	1	2	95	126
Wheat	7	1	0	2	9	7	10	12	6	5	59	3	5	1	2	2	9	8	6	8	11	55	114
Peas	1	2	3	2	1	4	4	12	7	4	40	4	2	2	3	2	7	2	14	3	4	43	83
Beans	4	2	1	1	7	1	1	4	5	3	29	1	0	1	4	0	9	4	6	5	9	39	68
Potatoes	1	0	1	0	1	2	6	16	10	7	44	2	2	0	0	2	4	4	4	3	4	25	69
Bran	4	2	2	12	9	5	17	7	10	3	71	4	0	2	3	3	2	2	0	1	2	19	90
Oats	2	2	1	2	11	4	3	9	8	5	47	4	1	8	8	3	20	8	14	12	17	95	142
Wheat	2	5	4	3	5	5	7	6	15	8	63	14	6	2	7	7	17	12	17	12	8	102	165
Peas	6	1	2	4	2	3	8	13	5	6	50	6	4	7	6	6	2	9	6	4	7	57	107
Beans	2	1	3	6	6	2	13	6	7	5	51	6	3	2	5	5	3	0	4	0	10	38	89
Potatoes	3	3	1	4	6	4	4	5	13	3	46	6	2	6	2	5	8	2	2	1	3	37	83

Fig. 2. Graph showing attractiveness of bait as obtained from trials in Lincolnshire, October and November 1925.

recorded. Since sifting was impossible in the time available the interspaces were carefully dug over, therefore the figures for wireworms in the interspaces and the percentage attraction cannot be regarded as strictly accurate though they give a good indication of the influence of the distance between the baits. In the case of the wheat, cold and frosty weather occurred during the period of baiting and this may have driven the wireworms deeper into the soil especially in the wider interspaces where no food was available. The results of this experiment, given below, indicate that it is undesirable to set bait rows further apart than 4 ft.; and in subsequent experiments the bait was usually drilled in rows 3 ft. apart, since the ordinary corn drill could be easily arranged to drill two rows at this distance apart.

Table VI.

Bait	Row No.	Distance apart ft.	Wireworms attracted	Wireworms in interspace	Percentage attracted
Beans	1	—	26	—	—
	2	2	17	3	85
	3	3	15	4	79
	4	4	12	6	67
	5	5	14	18	44
	6	6	10	21	32
Wheat	1	—	11	—	—
	2	2	15	1	94
	3	3	6	1	86
	4	4	10	1	91
	5	5	8	4	67
	6	6	11	8	58
	7	7	12	2	86
	8	8	12	7	63
	9	9	27	4	87
	10	10	25	4	86

On November 12th the area unoccupied by these various experiments, about $3\frac{1}{2}$ acres in extent, was well harrowed and drilled with wheat to test on a field scale the value of baiting in assembling the wireworms. The corn drill used was the usual type of horse-drawn machine, about 6 ft. 6 in. wide, and the intermediate spouts were cut out to leave two running about 3 ft. 3 in. apart. The wheat was planted about $2\frac{1}{2}$ in. deep.

A sudden change in the weather at this time resulted in almost continuous frost and some snow for a considerable time; and periodic examinations showed that only a few wireworms visited the bait. The trial was finally abandoned because in the spring, before the upward

movement of the wireworms in any numbers was apparent, it was necessary to prepare the field for the 1926 cropping.

Several lessons were learnt from these experiments. Potatoes, which are often advocated for trapping wireworms, proved much less attractive than other baits, especially germinating seeds. Bran, too, was not attractive for a long period and after about 10–12 days began to develop mould, which probably rendered it unpalatable to the wireworms. Peas, beans and large pieces of chopped potato were found to be unsatisfactory where baiting was to be followed by the use of an insecticide, for wireworms, particularly small ones, could be found tunnelling inside, where they were probably beyond the reach of the insecticide. The use of seeds which would germinate had a marked advantage over bait which would not grow, for after the period necessary to allow for the wireworms assembling, the bait rows could be easily traced by the green shoots which appeared above the ground. Since the wireworms attacked the young plants at the base of the plumule after the food stored in the grain was exhausted, where the pest was very numerous at the bait only a few green blades could be seen.

(2) *Field Experiments* 1926.

To test the conclusions arrived at by the foregoing experiments a field at Cottenham, Cambridgeshire, broken up in the autumn of 1925, was thoroughly cultivated to free the land of weeds, and worked down to a fine tilth. On April 13th it was drilled with wheat in rows 3 ft. apart and 2–3 in. deep. On April 23rd examination of 3 one-yard samples yielded a total of 58 wireworms and when the experiment was concluded 8 representative one-yard samples yielded 119 wireworms or about 15 per yard, which by calculation indicated an assemblage of about 60,000 wireworms per acre. The interspaces relative to the 8 samples taken yielded 24 wireworms, or about 14,500 wireworms not assembled. The attraction figure would therefore be about 80 per cent. This experiment indicated that under ordinary field conditions considerable numbers of wireworms can be assembled at a suitable bait.

Experiments were carried out at Worthing and Cheshunt to test the possibilities of baiting land soon after breaking up, since for crops like strawberries successful baiting would enable the plants to be set out a year earlier. The trials were made in March–April within a month after breaking up the land. The undecayed sods made the setting of the baits a difficult process; in the previous experiments it had been possible to use a drill for many of the baits, but in these experiments it was necessary to set the baits by hand. At Worthing a variety of baits were

planted in rows 20 yd. long and 3 ft. apart; and though the numbers of wireworms at the baits were low even after an exposure of 21 days, they give an indication of the relative efficiency of the different baits. Fourteen yard samples taken periodically between March 23rd and April 14th from each bait row yielded wireworms as follows:

Bait	No. of wireworms assembled
Wheat	45
Oats	24
Brewers' grains	15
Rape meal	10
Castor meal	7
Rape cake dust	9
Bran (wet)	8
,, (dry)	4
,, sweetened with sugar	7
,, sweetened with treacle	9
,, and wheat	11
Malt culms	4

It was interesting to note in this experiment that rape meal and rape dust, the bait usually recommended for attracting wireworms, proved very disappointing when compared with wheat and oats. Sweetening the bran did not increase its attractiveness to any extent, nor did mixing bran and wheat. This latter mixture has since been found very effective for short period baiting, but for long period baiting the mould development on the bran extends to the wheat and the bait no longer attracts the wireworms.

In the Cheshunt experiment wheat only was used as bait. It was planted on April 8th in rows 2 ft. apart and sampling on May 5th yielded 18 wireworms from 4 one-yard samples and on May 25th 7 samples yielded 39 wireworms or an average of about 5 per yard which, judging by the number of wireworms still present in the turf and the interspaces, was only a very small proportion of those present in the land.

The results from these two experiments showed that baiting could not be effectively carried out on new land until the greater part of the turf was broken down, a process requiring from 7 to 12 months, according to the character of the soil.

(3) *Experiments in Glasshouses* 1925–6.

In the winter of 1925, when the downward migration of the wireworms prevented further work on baiting being conducted in the open, it was decided to carry out trials in glasshouses in Hertfordshire where wireworms had caused serious losses to tomatoes during the previous growing

season. The land had been broken up for glasshouse culture in the winter of 1924 and had carried its first crop of tomatoes in 1925 but there was still a considerable amount of undecayed turf which somewhat hampered the work. The soil in house No. 1 was sampled during the first week in December and 37 wireworms occurred in 40 samples 6 in. in diameter and 18 in. deep. In many cases the samples were taken to a depth of 21 in. but no wireworms were found at a depth greater than 12 in., the majority occurring within the top 9 in. of soil. The house was baited on December 9th and 10th with wheat, castor meal, brewers' grains, rape meal, bran and rape cake dust. A different system of applying the baits was adopted in order to test the relative efficiency of the bait substances under other conditions. The bait was set in holes made with a bulb planter, 2–3 in. deep and 2 ft. apart each way. A plot containing 30 sets was allotted for each bait and the whole series was taken in duplicate. At the time of setting the baits the soil temperature was 36° F. and the air temperature 32° F. Periodic examinations of the bait were made, but it was not until January 4th, 1926, that the wireworms began to assemble. On that date, when the soil temperature had risen to 47° F. one sample in each plot was examined and wireworms were found at the wheat, bran and castor meal. A week later more wireworms occurred in the samples taken and after that the assemblage was rapid and continuous. The final examinations were made on February 9th and the following table gives particulars of the relative efficiency of the baits used and the total number of wireworms from each plot.

Bait	No. of sets	No. of wireworms assembled
Wheat	60	429
Brewers' grains	60	85
Castor meal	60	73
Rape meal	59	71
Bran	59	68
Rape cake dust	60	63

As in the field experiments, wheat, the only "live" bait, proved outstandingly attractive while the remaining baits were comparatively inefficient. The periodic examinations of the baits showed that the wheat steadily increased in attractiveness throughout the time of exposure. In this experiment the wireworms collected were recorded in three groups; of the total of 789, 42 per cent. were over 18 mm. in length, 36 per cent. were 10–18 mm. long and 22 per cent. were under 10 mm.

The soil of No. 2 glasshouse, which was rather cloddy and contained

much undecayed turf, was sampled and yielded 57 wireworms from 64 samples 6 in. in diameter. On February 3rd it was baited with wheat and brewers' grains, the two baits which had proved most attractive to wireworms in the preceding experiment. The baits were set alternately in rows, rows 1–5 inclusive being placed 2 ft. apart and rows 6–10, 3 ft. apart. At mid-day on the date of sowing the soil temperature was 48° F. Gentle heat was turned on in the house after baiting and when the baits were sampled after 20 days' exposure the following figures were obtained:

Bait	Distance between rows ft.	No. of 1-yard samples	Wireworms assembled	Approx. No. of wireworms per yard
Wheat	2	19	262	14
,,	3	12	193	16
Brewers' grains	2	12	45	4
,,	3	15	61	4

Totalling the figures for each bait, 31 yd. of wheat row yielded 455 wireworms or an average of about 15 per yd. and 27 yd. of brewers' grains row yielded 106 wireworms or about 4 per yd.

In a glasshouse near Waltham Cross a further experiment was conducted on lines similar to the above and it was found that wheat attracted approximately six times as many wireworms as bran, rape or castor meal.

These experiments showed that in glasshouses under suitable conditions wireworms will assemble in considerable numbers at baits; and that, as in the field, germinating seed is by far the most attractive bait yet tried. As was noted in the open, soil conditions have considerable influence on the efficiency of the baits, for baits set in soil having a temperature of 32° F. failed to attract the wireworms until the soil temperature rose above 40° F.

(4) *Baiting wireworms among growing crops.*

Since wireworms exhibited marked preferences for certain of the baits used in the foregoing experiments, and since examination of weeds in wireworm infested land in Lincolnshire showed that the pest occurred commonly at the roots of some species and only rarely at the roots of others in close proximity, it was thought if an attractive bait were set between rows of crop plants known to be suffering from attack by wireworms, it might be possible to attract the insects and thus save the plants. With the assistance of Mr Kent, Horticultural Superintendent, Isle of Ely, permission was obtained to test this idea in a field of strawberries in the Wisbech area, known to be infested with wireworms. The

land had been ploughed up in the autumn of 1924 and the strawberries which were planted out in the autumn of 1925 had not made good growth, many having been killed during the winter and others obviously suffering from wireworm attack. From the field 19 soil samples 6 in. square and 8 in. deep yielded 64 wireworms or by calculation about 586,000 per acre. On April 9th, 1926, wheat, oats, bran and malt culms were set as bait between the rows of strawberries. Periodic examinations showed that the wireworms were assembling in large numbers. All the baits proved highly attractive for the first fortnight; wheat and oats, especially the latter, were increasingly attractive during the third week but bran and malt culms showed a decrease in efficiency during that period. The following table shows the number of wireworms assembled at the various baits:

Date 1926 April	Bait	No. of 1-yard samples	Wireworms present	Average per yard
16	Wheat	4	30	7·5
22	„	4	47	11·75
30	„	4	48	12·0
16	Oats	4	35	8·75
23	„	4	62	15·5
26	„	4	114	28·5
16	Malt culms	4	41	10·25
23	„ „	4	35	8·75
30	„ „	4	20	5·0
16	Bran	4	41	10·25
23	„	4	41	10·25
30	„	4	33	8·25

In order to determine to what extent the wireworms were leaving the strawberry plants for the baits, 5 sections, each 1 yd. square and containing 4 strawberry plants, their relative interspaces and the bait row, were examined 3 weeks after the setting of the bait and the following results obtained:

		Wireworms assembled					
Plants	Section No. ...	1	2	3	4	5	Total
4 strawberry plants and interspace		1	1	3	3	0	8
1 yard wheat bait		7	5	8	2	7	29
4 strawberry plants and interspace		3	5	3	0	1	12
1 yard wheat bait		11	9	31	6	3	60

Thus in an area containing 109 wireworms 89 or 81·6 per cent. were attracted from the plants on which they were feeding to bait set in rows between the crop.

In May 1926 an experiment in baiting among crops was conducted on a flower farm in Cambridgeshire. On May 17th baits were drilled between rows of various kinds of flowers and examination on June 2nd gave the following figures:

Variety of plants	Bait	No. of 1-yard samples	Wireworms		
			Assembled	Interspace and at plant roots	Total
Pyrethrums interplanted with Gypsophila	Wheat	6	16	23	39
Pyrethrums	,,	3	2	1	3
,,	Rye	3	4	4	8
,,	Oats	3	3	—	3
,,	Bran	3	5	3	8
Gladioli	Wheat	3	12	3	15
Chrysanthemums	,,	3	17	8	25
Asters	,,	3	20	2	22
Statice	,,	3	6	1	7
Calendula	,,	3	3	5	8

These results show that out of a total of 138 wireworms present in the area, 88 or approximately 64 per cent. were attracted to the baits.

Vine borders in a glasshouse near Chislehurst were found to be heavily infested with wireworms which were seriously hampering the growth of the vines. Wheat and bran mixed 3 : 1 was set as bait on July 9th. The borders had previously been thoroughly watered and the soil temperature was 76° F. Examined 14 days later, 9 representative one-foot samples yielded 92 wireworms assembled and 4 wireworms in the relative interspaces, or an attraction of 96 per cent.

It is apparent from these experiments that baiting between growing crops has distinct possibilities as a means of attracting a considerable proportion of wireworms from the crop plants which are being attacked.

(5) *Conditions for successful baiting.*

From the experiments herein described on baiting for wireworms in arable fields, in glasshouses, and between growing crops it is obvious that certain conditions are necessary for successful results. The baits should be set just prior to or during the height of wireworm activity near the surface of the soil; that is in autumn during October and November, or in spring during March, April and May. The land to be baited should be cleared of weeds which by affording other food for the wireworms might detract from the efficiency of the bait. The bait should be selected with due regard to the time available for its exposure; bran

and wheat are both quick acting baits but the former tends to lose its attractiveness after about a fortnight; wheat and oats both remain attractive for 3 weeks or more, the latter being less attractive during the first few days.

III. Baiting and Calcium Cyanide for Wireworm Control.

From time to time various substances have been recommended as soil insecticides, and under ordinary farming conditions soot, salt, lime, kainit, various artificial manures, and naphthalene have been used and, though acting chiefly as repellants, they have occasionally been the means of temporarily relieving crops from wireworm attack. In 1922 calcium cyanide in various degrees of fineness became available in America and being a cheap and easily handled source of hydrocyanic acid gas, a potent insecticide, attracted the attention of economic entomologists, as a possible remedy for the control of soil insects; and Quale (15) published a brief note on this material as a soil fumigant in 1923. The first mention of calcium cyanide in connection with wireworm control was made by Horsfall (6) early in 1924 and since that time reports on its use against wireworms have been published by Campbell (2), Spuler (20) and Horsfall and Thomas (7). These investigators found that calcium cyanide was highly toxic to wireworms, and though rather expensive for field use, if it was used in conjunction with baiting was likely to prove a reasonable and efficient means of controlling this pest. Experiments conducted in various parts of England in 1925 and 1926 by the writers bear out the findings of the American workers.

Calcium cyanide, $Ca(CN)_2$, is made from calcium cyanamid, $CaCN_2$, which is produced by a process of fixation of atmospheric nitrogen. It is quite distinct from cyanamid, having an extra carbon atom and totally different characteristics. When calcium cyanide is acted upon by atmospheric water vapour or soil moisture, hydrocyanic acid gas is given off; the reaction is expressed thus:

$$Ca(CN)_2 + 2H_2O = Ca(OH)_2 + 2HCN.$$

When this takes place the residue is lime and certain nitrogenous products which are beneficial to vegetation. In granular form crude calcium cyanide is bluish grey in colour, and about 90 per cent. passes through a sieve of 20 meshes to the inch and 40 per cent. through a 30 mesh sieve. In 10 samples of the commercial form used in these experiments analysis showed a minimum of 46·20 per cent. pure calcium cyanide, a maximum of 53·12 per cent., and an average of 49·1 per cent.

(1) *Preliminary tests.*

In order to determine the range of influence of calcium cyanide, experiments were conducted with wireworms in copper gauze cages. These cages were filled with soil and 5 wireworms put in each. The cages were then placed in the soil in various positions relative to a row of calcium cyanide granules drilled at the rate of 3/8 oz. per yd., the whole experiment being carried out in duplicate. Five days after the experiment was set up, the following results were obtained:

	Wireworms					
	Dead		Alive		Moribund	
Position of cage	A	B	A	B	A	B
Upon cyanide	5	5	0	0	0	0
Beside cyanide	5	5	0	0	0	0
1 in. from cyanide	5	5	0	0	0	0
2 in. „ „	5	5	0	0	0	0
3 in. „ „	2	2	2	3	1	0
Control cage	0	0	5	5	0	0

This experiment indicated that the lateral range for high toxicity was 2–3 in., and a similar experiment with cages 2 in. to the side and from 1 to 3 in. above, and 1 to 3 in. below the cyanide indicated efficient killing within this range.

(2) *Field Experiments in Lincolnshire* 1925.

In this series four experiments were conducted. The wireworm infested land was first cleared of weeds and then lightly cultivated and baited. After varying periods of exposure, during which examinations for assembling wireworms were made, calcium cyanide was applied to the bait rows by means of a hand-drill (Pl. XXV, fig. 1) to which a special deep plough attachment (*A*) had been fitted, thus enabling the cyanide to be deposited about 4 in. deep, *i.e.* just below the level of the baits. The drill had an indicator, by which the aperture adjustment was set according to the size of the seed to be sown. When the indicator was fixed at "Salsify" it was found to drill 60 yd. of bait row with 1 lb. of granular calcium cyanide. After the application of the cyanide, the land was lightly pressed to cover the cyanide thoroughly and to close the larger soil interspaces.

Experiment No. 1 was designed to test the toxicity of calcium cyanide to wireworms under field conditions. On October 19th, 1925, after an

exposure of 14 days, 12 rows of bait were treated with calcium cyanide at the rate of 1 lb. per 60 yd. Rain had fallen the night before the application of the cyanide and the ground was therefore rather wet and somewhat adhesive, which interfered to some extent with even drilling. Four days after application the rows were sifted out and yielded a total of 299 wireworms, of which 143, or 47·8 per cent., were dead. From untreated rows from the check plot a total of 309 live wireworms were obtained.

Experiment No. 2 was laid down to determine the influence of depth of baiting on the killing efficiency of calcium cyanide drilled at a depth of 3½ to 4 in. The baits were set on October 7th, left undisturbed for 14 days and cyanide drilled at the same rate as in Exp. 1. Six days after treatment the rows were sifted out and the results, which indicated that 3 in. is the most satisfactory depth to set bait, were as follows:

Depth of bait in.	Wireworms			
	Alive	Dead	Total	% Killed
2	51	28	79	35
3	35	30	65	46
4	52	25	77	32

In similar but untreated bait rows, 150 wireworms, including 1 dead specimen, were obtained.

Experiment No. 3. In this test six bait rows after being undisturbed for 14 days, were drilled with calcium cyanide as above, to determine the influence of time and exposure on toxicity. The first three rows were examined 6 days after treatment and the second three 12 days after treatment. The results showed 77·7 per cent. killed after 6 days and 78·5 per cent. after 12 days, indicating that under field conditions, calcium cyanide is likely to kill the majority of the insects within 6 days after application.

Experiment No. 4. Fifty rows of wheat 10 yd. long and 4 ft. apart were drilled on October 26th and 27th; on November 9th and 10th, calcium cyanide at 20 different rates between 34 lb. per acre (approximately 1 lb. per 100 yd.) and 143 lb. per acre was applied. The series was put down in duplicate and 10 rows receiving no calcium cyanide were left as a check plot. Examination 7 days after treatment yielded the results given in the accompanying table. From this it will be seen that a dressing of 73 lb. per acre, or 1 lb. per 50 yd. of bait row, gave over 90 per cent. kill of assembled wireworms and dressings over this amount gave, with one exception, kills of at least 95 per cent.

Weight of calcium cyanide in lb. per acre	Wireworms			
	Alive	Dead	Total	% Killed
34	8	15	23	68
39	6	21	27	77
45	7	34	41	82
51	10	27	37	71
56	16	38	54	71
62	10	53	63	83
68	10	54	64	82
73	5	52	57	91
79	3	48	51	96
85	4	37	41	85
90	2	61	63	96
96	0	52	52	100
102	2	35	37	95
107	1	36	37	97
113	2	29	31	95
119	1	41	42	98
124	0	67	67	100
130	2	59	61	97
136	1	60	61	98
143	0	42	42	100
Nil	137	0	137	0

(3) *Experiments in Glasshouses*, 1926.

At Hatfield a glasshouse, known to be heavily infested with wireworms, was baited on February 3rd, and the house gently heated. The soil was cloddy and when calcium cyanide at rates varying from 2–3½ lb. per 175 yd. was applied 14 days after baiting, these clods hampered the smooth running of the drill, hence the distribution of the cyanide was not quite even. Six days after treatment siftings of representative yard samples yielded results as follows:

Bait yards examined	Calcium cyanide per row of 175 yards lb.	Wireworms				
		Alive	Dead	Comatose	Total	% Killed
10	2	25	40	14	79	50
12	2½	36	87	9	132	66
12	3	72	52	5	129	40
12	3½	28	70	8	106	66

A wireworm-infested glasshouse at Worthing was baited with wheat sown 2–3 in. deep in rows 2 ft. apart on February 24th, and gentle heat turned on in the house. Fourteen days later calcium cyanide at varying rates was applied, the rows of bait, readily located by the young shoots

of the wheat, being carefully followed with the drill. Six and seven days after the application of the cyanide, siftings yielded the results given below:

Amount of calcium cyanide per 150 ft. of bait row lb.	Wireworms		
	Alive	Dead	% Killed
¾	65	117	64·28
1	51	195	79·26
1¼	22	111	83·45
1½	9	144	94·11
1¾	12	149	92·54
2	19	141	86·50
2½	0	151	100·00
Nil	232	0	0

(4) *Experiments amongst growing crops*, 1926.

Two experiments were conducted amongst growing crops to determine whether calcium cyanide could be used to control wireworms without causing injury to the crop.

A strawberry plantation at Wisbech, baited on April 8th, was dressed with granular calcium cyanide on May 4th at the rate of 2½ lb. per 50 yd. of bait row. Examination of 10 one-yard samples 3 days after treatment yielded 59 wireworms, 1 was alive, 2 were moribund, and 56, or 95 per cent., were dead.

Vine borders under glass at Chislehurst were treated, after baiting, with calcium cyanide at the rate of 3 lb. per 50 yd. of bait row. Examination 3 days later gave a total of 97 wireworms including 92, or 95 per cent., dead specimens, from 11 separate one-foot samples.

These experiments are of special interest because they show that wireworms amongst growing crops can be controlled with calcium cyanide, for in neither case were the plants injured by the treatment.

(5) *Cost of treatment.*

The cost of treating land for the control of wireworms by the baiting and calcium cyanide method will depend on the value of the land and the type of crops to be grown. The spacing of the bait rows will be governed by the cropping, *e.g.* in glasshouses where a wireworm may cause the death of one or more tomato plants each capable of bearing 6–8 lb. of fruit, the bait rows should be close together so as to attract as many wireworms as possible. In such cases the cost of treatment will be higher because of the extra bait required, the longer time to

set the bait rows and the greater amount of calcium cyanide required. In the case of a block of tomato houses each 150 ft. long by 26 ft. wide, accommodating 12 rows of bait 2 ft. apart, each house would require 1½ st. of wheat costing about 2*s*. 6*d*. and with a hand-drill this would take about an hour and a half to set. The cost of baiting each house, therefore, would be about 4*s*. Calcium cyanide at the rate of 1½ lb. per 150 ft., or 18 lb. for the house, would be required, and cost about 1*s*. 2*d*. per lb., or 21*s*. for cyanide with a cost of application of 1*s*. 6*d*., making a total cost of 22*s*. 6*d*. for each house. This figure is interesting when compared with the methods usually adopted in these houses, which is to trap the wireworms with broccoli stems set in the ground to a depth of about 6 or 8 in., the woody base of the stalk and the roots protruding and serving as a handle for use when examining the traps. The broccoli stems are set between the rows of tomato plants usually about 2 ft. apart either way and are examined periodically, the wireworms boring into the stems being removed and destroyed. The cost of treatment in this manner worked out as follows for each house, the men being paid at the rate of 1*s*. per hour:

Preparing and setting bait, 1 man 2 hours	2*s*.
Examining baits, 4 men 3 hours, twice weekly for 4 weeks...	96*s*.

This treatment for each house, therefore, cost 98*s*. or about four times the cost of the baiting and calcium cyanide treatment. In some cases carrots are used as traps and under such circumstances, there would be the further cost of these to be added.

On agricultural land with wheat as bait drilled in rows about 3 ft. apart, 3½ st. is sufficient for an acre and about 90 lb. of calcium cyanide would be required, costing about £4. 10*s*. This, as an initial cost seems high but it must be remembered that the treatment will destroy wireworms which would otherwise be active and destructive for at least 3 years. The cost per year therefore over this period is not great, and in any case where intensive agriculture is followed, treatment at this expense might be adopted with advantage, though for general farming the cost might be considered prohibitive. An advantage of the baiting system is that by examining the bait it is possible to locate the main areas of wireworms infestation and only the infested portions of the field need be treated with the calcium cyanide.

IV. CONCLUSIONS AND RECOMMENDATIONS.

Experimental work on the control of wireworms in various parts of England and in connection with both agricultural and horticultural crops indicate that a successful means of control consists of baiting infested land with wheat and oats, or a mixture of wheat and bran, and when the wireworms have assembled in the bait rows, applying calcium cyanide to the rows by means of a suitable drill. This method can also be used among growing crops when the space between the rows allows room for baiting. During the course of the work much experience has been gained as to the most suitable method of carrying out the process, and based on this the following tentative recommendations are made. The land should be cleared of weeds and the soil worked to as fine a tilth as possible a week or so before the bait is set. The bait, preferably wheat or oats, should be sown 2–3 in. deep in rows 2–4 ft. apart according to the value of the crop to be grown and the extent of wireworm infestation. The bait rows should be examined at about four-day intervals to note the rate of assembling of the wireworms, because the time of application of the calcium cyanide will depend on this. Under ordinary conditions maximum assemblage will take place in about a fortnight but should cold weather intervene a week or ten days longer may be necessary. The calcium cyanide can be satisfactorily applied with a hand-drill of the "Planet" Junior or "Norrahammar" type, fitted with a deep plough attachment, depositing the cyanide at a depth of about 4 in., *i.e.* below the level of the bait. About 2 lb. of calcium cyanide per 100 yd. of bait row is usually sufficient to ensure over 75 per cent. kill of assembled wireworms under ordinary field conditions. Under glasshouse conditions, where 100 per cent. kill is most desirable, 3 lb. per 100 yd. should be used. The calcium cyanide should be covered with soil immediately after application and the land rolled or pressed to close the larger air spaces and prevent the rapid escape of the hydrocyanic acid gas. Six or seven days after treatment the land can be lightly cultivated and opened up to liberate any residual fumes and plants can be set or seed sown within the next two days.

Spring seems to be the most satisfactory time of the year to adopt measures for the control of wireworms for the soil temperature is steadily rising and the wireworms becoming more active after the winter period and working back into the surface soil for feeding purposes. In the autumn, though numbers will assemble at baits, the steady fall of the soil temperature gradually induces a cessation of feeding and causes

the majority of the wireworms to migrate deeper into the soil. There is also risk of a sudden period of cold weather occurring during the exposure of the bait and driving the wireworms down immediately, so that the labour of baiting is wasted. If treatment must be carried out in autumn, it is best carried out in late September or early October. Calcium cyanide should not be applied when the land is wet, for drilling is difficult, even distribution almost impossible, and the drill constantly clogs, and, moreover, the hydrocyanic acid gas does not permeate the soil effectively. Land can be baited with success 7 or 8 months after breaking up from grass or ley; before this time has elapsed, the presence of turf annuls the influence of the baits. If such land must be treated for wireworms, calcium cyanide can be applied by means of a hopper fixed to the beam of an ordinary plough and regulated to sow the insecticide just in front of the falling furrow slice. Pl. XXV, fig. 2 illustrates the type of apparatus.

Summary.

A study of the wireworm problem in Great Britian shows that this pest is especially important in areas devoted to intensive agriculture and commercial horticulture.

In addition to most farm crops, strawberries, various flowers and tomatoes are particularly susceptible to wireworm attack and because these crops so often occur on new land, losses due to the activities of the pest are frequent and serious. The nature and extent of wireworm attack, both in the field and in glasshouses, is described herein.

Examination of weeds growing in infested fields indicate that wireworms are found in numbers at the root of some plants such as couch-grass, *Agropyrum repens*, and yarrow, *Achillea millifolium*, while very few occur at the roots of plants like chickweed, *Stellaria media*, goutweed, *Chenopodium album* and annual nettle, *Urtica urens*.

Field observations on the movements of wireworms in the soil throughout the autumn, winter and spring indicate that there is a definite downward migration in autumn and an upward migration in spring. Correspondingly wireworm activity in the surface soil was noted to be at its height in September and October, and March, April and May.

Experiments on the use of baits as a means of assembling wireworms, indicated that large numbers will collect to baits such as wheat, oats, and bran, and moreover these baits can be used to attract wireworms from the roots of crop plants, on which they are feeding. Because of

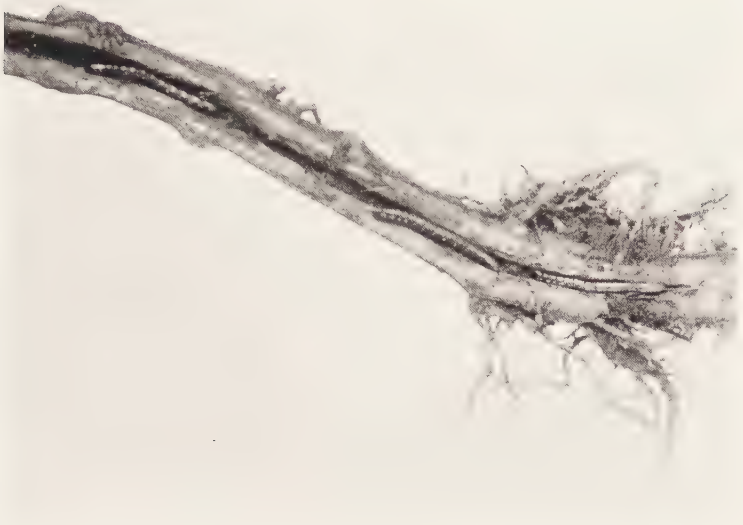


Fig. 2. Tomato stem showing damage by wireworms; three *in situ* (natural size).

Fig. 1. Potato tuber showing typical wireworm injury ($\times \frac{3}{4}$).

Fig. 1. "Planet Jr" hand-drill with deep plough attachment (*A*) as used for application of calcium cyanide

Fig. 2. Plough with plough-applicator attached as used for application of calcium cyanide at the time of ploughing.

the seasonal movements of wireworms, baiting is best carried out in autumn or spring.

Calcium cyanide in granular form has been found to be highly toxic to wireworms and used, in connection with the baiting, at the rate of 2–3 lb. per 100 yd. of bait row, destroyed 75–100 per cent. of the wireworms assembled.

Based on the results of experiments conducted in Lincolnshire, Hertfordshire, and West Sussex, during the years 1925 and 1926, and involving 10,000 wireworms, recommendations are made for the control of wireworms by means of baits and the use of calcium cyanide.

BIBLIOGRAPHY.

(1) Board of Agriculture (1920). *Misc. Publ.* No. 23, p. 9.
(2) CAMPBELL, R. A. (1924). *Journ. Econ. Ent.* XVIII, 562.
(3) CURTIS, J. (1860). *Farm Insects.* London.
(4) FORD, G. H. (1917). *Ann. App. Biol.* III, 97.
(5) HAMMOND, J. (1912). *Journ. Agric. Sci.* IV, 386.
(6) HORSFALL, J. L. (1924). *Journ. Econ. Ent.* XVII, 160.
(7) HORSFALL, J. L. and THOMAS, C. A. (1926). *Journ. Econ. Ent.* XIX, 181.
(8) LEES, A. H. (1916). *Ann. Rept. Long Ashton Res. Stn.* p. 37.
(9) MIALL, H. W. (1902). *Injurious and Useful Insects.* London.
(10) MILES, H. W. and PETHERBRIDGE, F. R. (1927). *Journ. Min. Agric.* XXXIII, No. 11, p. 931.
(11) ,, (1927). *Agricultural Progress.* IV, 11.
(12) Ministry of Agriculture (1923). *Misc. Publ.* No. 39, p. 7.
(13) MORRIS, H. M. (1922). *Ann. App. Biol.* IX, 2821.
(14) ORMEROD, E. A. (1890). *Manual of Injurious Insects.* London.
(15) QUALE, H. J. (1923). *Californ. Univer. Coll. of Agric. Rept.* 1922–3.
(16) ROBERTS, A. W. R. (1919). *Ann. App. Biol.* VI, 116.
(17) ,, (1921). *Ibid.* VIII, 193.
(18) ,, (1922). *Ibid.* IX, 306.
(19) ROEBUCK, A. (1924). *Journ. Min. Agric.* XXX, No. 2.
(20) SPULER, A. (1925). *Journ. Econ. Ent.* XVIII, 702.
(21) TATTERSFIELD, F. and ROBERTS, A. W. R. (1920). *Journ. Agric. Sci.* X, 199.
(22) THEOBALD and MCGOWAN (1916). *Journ. Board of Agric.* Supplement, No. 15.
(23) THOMPSON, M. (1924). *Ann. App. Biol.* XI, 349.
(24) WARDLE, A. R. and BUCKLE, P. (1923). *Principles of Insect Control.* Manchester.

(*Received January 27th*, 1927.)

REVIEWS

The Biological Relations of Optically Isomeric Substances. By ARTHUR R. CUSHNY. Baillière, Tindall and Cox. Pp. vi + 80. $2.00.

This small book, the product of the Dohme Memorial Lectureship, and the last published work of the late Prof. Cushny, makes profoundly stimulating reading. The peculiar value of such lectureships as these is, that they constitute a platform from which acknowledged masters in various fields of scientific endeavour can present a fairly complete view of some of the problems met with in the course of their work, and enable a partial synthesis to be made of results of researches scattered through many journals. Their influence in many cases spreads into new fields. It appears to have been the hope of Prof. Cushny that this should be the case, for whereas this borderland subject has proved of a certain interest to chemists and of importance to the pharmacologist, the biologist, in general, has held almost entirely aloof from the consideration of the bearing optical activity may have on the phenomena exhibited by living matter. This, perhaps, is the more remarkable in view of the fact that Pasteur, from a starting point dealing with the crystallographic relationships of the optical isomers of tartaric acid, found them a direct pathway to his great work in biology and medicine.

The admirable historical introduction may prove to the purely biological reader as fruitful a portion of the book as any. The selective synthesis of optically active compounds and their origin in nature, which have an interest in the evolution of living matter, is dealt with succinctly and suggestively. This naturally leads to the selective action of enzymes and the preferential growth of micro-organisms on one of the pair of isomers—the well-known lock and key effect of the biochemist, and to the decomposition of the isomers in living tissues. To the general physiologist these facts constitute a body of important knowledge, but a definite query arises as to whether the mycologist and bacteriologist have exploited this field of work as critically and thoroughly as it unquestionably deserves, for here are processes susceptible of exact quantitative measurement which, at first sight, would seem to be susceptible to the differentiation of strains.

Of necessity the subject-matter is mainly devoted to an account of the relationship existing between optical activity and configuration on pharmacological and toxic action. But here again the author takes as wide a view of the matter as possible, and some attempt is made to draw parallelisms between this phase of the subject and other biological effects indicated above and certain well-known biochemical methods of preferential precipitation. Analogies of this order are not dealt with in any uncritical way and throughout the book individual researches and opinions are evaluated with considerable spirit.

These lectures also contain an entirely welcome reminder of the difficulties of applying some one chemical or physical concept to pharmacological and physiological action, and that the physiological activity of a chemical substance is seldom purely chemical or purely physical.

The lectures end on the note that the methods required for further advance are rather those of chemistry than biology. The reviewer feels, that however true this may be from a purely pharmacological aspect, a separation into watertight compartments of workers in this field would be little short of disastrous.

Prof. Cushny's expressed aim in choosing the subject of these lectures was to attract to it more attention and lead others to advance it further. On closing the book one regrets deeply that his untimely death has removed a pioneer guide and powerful influence to this end.

F. TATTERSFIELD.

Enzymes: Properties, Distribution, Methods and Applications. By Selman A. Waksman and Wilburt C. Davison. London: Baillière, Tindall and Cox, 1926. Pp. xii + 364. 25*s.* net.

This volume does not pretend to be an original contribution to the subject but is the outcome of an endeavour to collect in as concise a form as possible the available information in regard to enzymes and to indicate the sources from which more detailed knowledge may be obtained. With this aim the authors have consulted over 2000 papers on the subject and pieced together the irregular and loosely fitting fragments into some kind of a coherent mosaic. Particular attention has been paid to the occurrence and preparation of enzymes, to the methods of measurement, and to the practical applications of enzymatic activity—phases of the subject that are frequently overlooked in volumes on plant and animal physiology and even in more specific treatises.

The book is divided into four portions. The first, dealing in general with the properties of enzymes, contains an historical introductory chapter followed by chapters on enzymes in biological processes; the chemistry of enzymes and enzymatic reactions; factors which influence enzymatic reactions. The second portion deals in general with the distribution of enzymes and contains chapters on enzymes of the human and animal body; plant enzymes; enzymes of micro-organisms. The third portion discusses methods for the preparation and study of enzymes, containing chapters on the preparation, preservation, purification and measurement of enzymes; enzymes acting upon fats and esters (esterases); enzymes acting upon carbohydrates and their derivatives; enzymes acting upon proteins and their derivatives; oxidases and oxido-reductases; zymases; catalase. The fourth portion consists of a single chapter on the practical applications of enzyme activity. There follow 78 pages of references and an Index. The whole value of a book of this nature is in the ready availability of its data and this to a very large extent depends upon its Index. In the present volume the Index is not nearly detailed enough and does not give a fair idea of the mass of data the volume contains: its incompleteness seriously impairs the practical use of the book for reference. The volume is one for personal possession and the insertion of one's own annotations and a drawback to this is the price. For a lightly bound book of 364 pages of almost straightforward type-setting plus a few graphs, 25*s.* is too high a price and will prevent many who would find it very useful in their work from purchasing the volume.

The amount of literature in these borderline regions is becoming so vast that unless workers are, from time to time willing to sacrifice time and energy in collating scattered work and making it available in such form as this, scientific research will be crushed by its own weight and incoherence. In the present case an associate professor of soil microbiology in an Agricultural Experiment Station and an associate professor of pediatrics in a famous Medical School have combined to this end. Such a collaboration is indeed a promising sign of the times, and it is to be hoped that it foreshadows a bridging of the gulf between medical men and workers in the cognate branches of agricultural science. Both have much to gain and nothing to lose by a pooling of their knowledge and ways.

WILLIAM B. BRIERLEY.

The Composition and Distribution of the Protozoan Fauna of the Soil. By H. Sandon. Edinburgh and London: Oliver and Boyd, Biological Monographs and Manuals, 1927. Pp. xv + 237 with 6 Plates, 11 Tables, 2 Text-figures and 3 Charts. 15*s.* net.

A new chapter of interest in soil microbiology and in the protozoa was opened in 1909 when Russell and Hutchinson advanced their hypothesis on the part played by the protozoa in the life of the soil. They showed that partial sterilisation of soil by heat or volatile antiseptics was followed by large increases in the numbers of

ammonifying bacteria. In seeking for an explanation of this rise in bacterial numbers they discovered that the protozoa normally present in an untreated soil had completely or almost completely disappeared from the partially sterilised soil. A limiting factor had been removed and since some of the protozoa were known to ingest and feed upon bacteria when living in cultures, the suggestion was made that such protozoa might function in the soil as the factor limiting bacterial numbers.

Zoologists had known for many years that several kinds of protozoa could be found in fresh water and on moist vegetation, mosses for example, close to the soil surface but it was not known that they were to be found normally inhabiting arable and pasture soils. Moreover it was a distinctly novel and striking suggestion that they were probably functioning in the soil as consumers of bacteria and therefore limiting its fertility.

Many questions concerning them and their life in the soil called for answers and most promising lines of biological investigations were opened up. We need not go into details of all these but consider some that are relevant to the present purpose. Were the protozoa world-wide in their distribution and how far down in the soil could they be found? Did they occur in all kinds of soil and what kinds of Protozoa were to be found? Were they entirely new to science or did they belong to genera and species already known? These questions are discussed in the book under review which is the latest to be added to the admirable series of Biological Monographs and Manuals. The book is divided into two parts. The first 69 pages are devoted to the general biology of the soil protozoa considered under various aspects whilst the remainder is purely zoological and consists of brief descriptions of all the protozoa so far recorded from soil systematically classified.

In the opening section methods for the study of soil protozoa are first given. Then follow notes on the large collection of soils from various parts of the world which have been examined for their presence. The results of this examination are set out in tabular form along with figures for total nitrogen, moisture content and *p*H values. Under the head of "Influences affecting Protozoan Fauna of Soils" a number of very interesting points are discussed such as geographical influences, rainfall, influence of vegetation, irrigation, soil type, soil bacteria, vertical distribution of protozoa in the soil and physical and chemical properties of the soil. Correlations between the different groups of protozoa and between the numbers of protozoan species and nitrogen content, hygroscopic moisture and *p*H value are also examined. All this is simply written and in a well-balanced manner particularly the concluding discussion, pp. 55–58. The only criticism we have to offer of the first part of the book is that it is made somewhat difficult to read and carry in the mind because of the way in which it is set out. The reason being that the same weight of type is used throughout for cross-headings whether for principal or for subsidiary sections. It is probable that the matter could have been more suitably presented to the reader by throwing it into chapter form with appropriate sectional and subsectional headings.

Introducing the zoological part of the work there is an account of the soil protozoal community in which it is shown that the majority of the 250 species so far found in the soil have been recorded from other habitats also, whilst 21 species only belong peculiarly to the soil.

The descriptions of the species are brief, adequate and to the point and after each main group, Mastigophora, Rhizopoda and Ciliata there is a very full table, bewildering one had almost said, to facilitate the running down of any given species belonging to the group.

In bringing so much scattered information into a handy form the book will be indispensable to the investigator in soil microbiology and to the general biologist desirous of studying the subject. Its value would, we think, have been even greater had it been possible to find room for more drawings of protozoa. The six plates are good and the figures clear and not crowded whilst particularly useful is Plate I showing the appearance of the cysts of some of the commoner forms, but we should have liked to see more figures even had it meant sacrificing one or two tables or possibly the three charts at the end of the book. The time and labour expended on the latter might, we feel, have been devoted to giving us more drawings.

There is a good bibliography and the whole work is happily almost completely free from errors or misprints. We have, however, noted the following: p. 73, Plate IV should be Plate II and p. 186, Bütochli is used in error for Bütschli.

T. GOODEY.

Hydrogen Ion Concentration. Its Significance in the Biological Sciences and Methods for its Determination. Volume 1. Principles of the Theory. By LEONAR MICHAELIS. Authorised translation from the second revised and enlarged German edition, by W. A. PERLZWEIG. London: Baillière, Tindall and Cox, 1926. Pp. xiv + 299. 32 Figures. 22*s*. 6*d*.

Michaelis has been one of the pioneers in the application of physico-chemical methods to the study of biological systems. His *Wasserstoffionenkonzentration*, published in 1912, was the first and for many years the only comprehensive treatment of the most fruitful section of this work—the definition and measurement of the intensity factor of acidity and alkalinity. The subject developed so rapidly both on the theoretical and applied sides that the second edition was planned in three separate volumes dealing respectively with theoretical foundations, the methods of measurement and the applications, especially to colloid chemistry and physiology. Volume I on "The Principles of the Theory" was published in German in 1921, but the translation, here reviewed, includes a few brief discussions of some of the more significant advances in the intervening five years. Unfortunately Volumes II and III have not yet appeared. Although these applied sections may appeal more directly to the biologist, some acquaintance with the theoretical principles is clearly essential if measurements of the complex systems of biology are to be accurately performed and correctly interpreted. In the first half of the book the chemical equilibria of the ions are presented clearly and in detail. The second half on the ions, especially the hydrogen ions, as sources of electrical potential differences is more novel; an attempt is made to build up into a single coherent structure a series of facts and theories derived from isolated physical, chemical and physiological investigations of these phenomena. Unity of treatment has been maintained at the expense of the literature references.

In Chapter I the results of the application of the simple mass action law to the electrolytic dissociation of water and weak acids and bases are brought out with particular clearness by means of the extensive use of dissociation and dissociation-residue curves. These lead easily to the amphoteric electrolytes and the concept of the isoelectric point. The influence of the hydrogen-ion concentration on the solubility of slightly soluble compounds of special biological interest, *e.g.* calcium carbonate and uric acid, is treated in detail. It may be noted that the translator follows the convenient American convention of writing Sørensen's symbol as *p*H.

The significance of titratable acidity, *p*H value and buffer action in the measurement of acidity are briefly discussed. Chapter III forms a particularly useful introduction to the most difficult aspect of the ionic theory—the "anomaly of strong electrolytes." Neutral salts do not obey the simple mass-action law, if their degree of dissociation is estimated, as in the case of weak electrolytes, by means of the electrical conductivities of their solutions. They are now regarded as completely dissociated but influenced by electrical interionic forces and hydration. Further they appear to increase the degree of dissociation of weak electrolytes, so that in physiological processes the absolute dissociation constants of the weak electrolytes will need to be replaced by constants reduced to the physiological salt contents. Michaelis's treatment is essentially historical, but it is sufficient to show where caution is necessary and to indicate the nature of the modifications that the newer theories may introduce.

The effects of a possible actual salt formation and electrolytic dissociation in non-aqueous solutions are briefly considered in Chapters IV and V.

Although all solutions of electrolytes contain equal amounts of positively and negatively charged ions, when considered in bulk, slight inequalities may occur at the boundaries of different phases. The large amounts of free electricity involved in such separations of the oppositely charged ions give rise to a number of electrical phenomena which may have important physiological bearings. The potential differences between metallic electrodes and solutions (Chapter IV) provides the basis for the electrometric estimation of hydrogen ion concentration by the hydrogen or the quinhydrone electrode. Diffusion potentials (Chapter VII) occur at the junction of two aqueous solutions of different composition, but they are of little physiological significance and may easily be eliminated by the potassium chloride bridge in electrometric *p*H measurements. Chapter VIII describes the potential differences that arise at the boundary of two phases in consequence of the limitation imposed by electrostatic effects on the distribution of the ions between the phases, as in Beutner's oil-water chains, and Chapter IX those between solutions separated by a membrane impermeable to one of the ions, the Donnan effect.

A lengthy final chapter attempts to bring order out of the confusing subject of adsorption. No fundamental contrast exists between the so-called chemical and physical forces involved in chemical union and adsorption. The exceptional ease of adsorption of the hydrogen and hydroxyl ions leads to the classification of substances, not in state of molecular dispersion, as acidoid, basoid, and ampholytoid according as they show analogies with acids, bases and ampholytes. Electrokinetic processes such as the movement of suspended particles and the passage of water through a diaphragm under the influence of applied voltages are discussed in considerable detail in connection with the adsorption of ions. The distinction between these adsorption potentials and the true phase boundary potentials is emphasised.

This book will undoubtedly take its place as an indispensable reference book to be used in conjunction with a practical treatise such as Clark's *Determination of Hydrogen Ions*, by all workers, whether biologists or chemists, concerned with the better understanding of the chemical processes in living organisms and their environments.

E. M. CROWTHER.